DESIGN AND EVALUATION OF MICROSPONGE DRUG DELIVERY OF PSORALEN - ISOLATED FROM *Psoralea corylifolia*

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Mrs.K.Reeta Vijaya Rani, M.Pharm. (Ph.D),
Assistant Professor,
Department of Pharmaceutics,
Periyar College of Pharmaceutical Sciences for Girls,
Trichy – 620021.

CERTIFICATE

This is to certify that the dissertation entitled
"DESIGN AND EVALUATION OF MICROSPONGE DRUG DELIVERY OF PSORALEN – ISOLATED FROM *Psoralea corylifolia*"
submitted by ***S. Eugine Leo Prakash, B.Pharm*** to The Tamilnadu Dr.M.G.R Medical University, Chennai in partial fulfillment for the award of the degree of **MASTER OF PHARMACY** is an independent bonafied work of the candidate carried out in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Trichy-21, during the academic year 2007 - 2008 under my direct guidance and supervision and to my full satisfaction.

Place: Trichy-21.
Date:

Dr. R.Senthamarai, M.Pharm, Ph.D.,

Principal,

Periyar College of Pharmaceutical Sciences for Girls,

Trichy – 620021

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I recommend this Research Work for acceptance as Project for the partial fulfillment of the degree of **"MASTER OF PHARMACY"** of the Department of Pharmaceutics, Periyar College of Pharmaceutical Science for Girls, for the year March 2008.

Place: Trichy-21.

Date: **(Dr.R.Senthamarai)**

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B.Pharm

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INTRODUCTION

India is perhaps the largest producer of medicinal herbs and is rightly called the 'Botanical Garden of the World' there are very few medicinal herbs of commercial importance, which are not collected or cultivated in this country. Medicinal herbs have been in use for thousands of years, in one form or another, under the indigenous system of medicine like Ayurveda, Siddha, Unani, since independence in 1947, India has made tremendous progress in agro technology, process technology, standardization, quality control, research and development etc.

Out now 2,25,000 species of plants known, about 35,000 are claimed to have medicinal properties. Today's estimation indicate that about 80% of people in developing countries still on traditional medicine based largely on species of plants and animals for their primary health care. About 30% of the world wide sales of drugs are based on natural products.

India is one of the world's 12^{th} leading biodiversity centers with the presence of over 45,000 different plant species. India's diversity is unmatched due to the presence of sixteen different agro climatic zones to vegetation zones, 25 biotic provinces & 426 biomes (habitat of specific species).

India has 15,000-18,000 species of flowering plants, 2500 algae, 23000 fungi, 1600 types of lichen, 1800 varieties of bryophytes and an estimated 30 million types of microorganisms.

Of these about 15,000 - 20,000 plants have good medicinal value. However, traditional communities use only about 7,000 - 7,500 for their medicinal values.

The siddha system of medicines uses about 600, Ayurvedha 700, Amuchi 600, Unani 700 and modern medicines about 50.

According to 2004 United Nations Development Project (UNDP) report, the annual value of medicinal plants derived from developing countries is about $ 60 billion. There are 78 major modern plant based drugs on world market & the predicted 450 more potential drugs have an estimated of $ 180 billion.

Out of the large number of plants with known medicinal value and history of centuries of use in traditional medicine very few have been exploited in modern medicine. The main reason being lack of standardization, isolation and characterization of active principle. Recently World Health Organization (WHO) has taken an official interest on indigenous system of plant used particularly in natural sources like plants and animals[1].

USEFULNESS OF PLANTS IN THERAPY

Many of the early plant based drugs such as curare as a muscle relaxant, Quinine from *cinchona* for malaria, Reserpine from *Rauwolfia serpentina* for hypertension, Digitoxin from *digitalis* for heart ailments, the whole range of steroids from diosgenin which is obtained from *Discorea* tubers, Vincristence/vinblastine from *Catharanthus roseus,* the toxoids from *Taxus brevifolia* and *Taxus baccata* as antimalarial are all still in use. A number of new products are in the market or under development for indications as wide ranging as flue, herpes, dermatitis, wound healing, cancer, diabetes and **Leucoderma.**

APPROACH TO USE OF PLANTS IN MEDICINES

The Indian, Chinese, Tibetan and other Oriental systems use plants as medicines, as whole plants or as their extracts. These systems also use multiple plants in their galenic forms with very elaborate processing, often times custom-made, for specific disease condition and patients. This approach has been justified by invoking immunomodulation as a possible mechanism of action with multiple plant component and synergistic fashion to ensure therapeutic efficacy and minimal toxicity; plant and plant based drugs are non-toxic and have acceptable side effects.

PLANTS AS SOURCES OF ACTIVE MOLECULES

An approach comparable with the current new discovery process is to use plants as source of active molecules this approach has led to successful development of many molecules. Many of them in common use even today **Psoralen**, Strychnine, Brucine, Reserpine, Quinine, Quinidine, Ajmaline, Vinca alkaloids, Taxol, Artemesinin & a host of other products have come through this route. A number of semi-synthetic drugs have become major drugs for many disease conditions. Thus the entire fields of hormonal steroids have come out chemical modification of diasgenia, a product isolated from *Dioscorea tubers* or the *Mexican yam*.

The Chinese product Artemesinin has been converted to less toxic artmether & artether. In all these cases, the discovery of new derivatives was made possible only due to the fact that original plants have shown activity, even if it was low & hence was only a lead.

PLANTS AS LEADS TO DISCOVERY OF NEW DRUGS

Based on the strong and traditional knowledge based on the use of plants as therapeutic agents a rational approach is being developed to use medicinal plants as a lead for the discovery of active molecules.

Established drugs are also used as leads to synthesize new derivatives, which led to the well known and often unreasonably maligned me-too category of drugs. They are also termed as molecular manipulations or molecules roulette of existing drugs[2].

INDIAN SYSTEM OF MEDICINE

"Indigenous medicine" is used to refer to a variously defined body of knowledge that is concerned with healing but which can take many different forms. It can refer to the use of herbs and plants found locally and in highland Tigray as a

drink, salve or inhalant; bloodletting; bone-setting; cauterization; the utterance or writing of special prayers for curing purposes; exorcism of spirits said to possess the body; and the use of holy water and other sanctified substances such as soil, ash, or sand. Different indigenous healers specialize in one or more of these types of healing.

Indian system of Medicine and Homoeopathic (AYUSH) cover both the systems which originated in India and outside but got adopted in India in course of time. These systems are Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homoeopathy. People are getting inclined for treatment through these systems due to lesser side effect in comparison to the modern medicines.

Ayurveda System of Medicine

Ayurveda means the "Science of Life". The knowledge Ayurveda was comprehensively documented in Charak Samhita and Sushruta Samhita. Ayurveda takes an integrated view of the physical, mental and spiritual and social aspects of human beings, each impinging on the others. The philosophy of Ayurveda is based on the theory of Panchmahabhutas (five-element theory) of which all the objects and living bodies are composed of. The combination of the five elements are represented in the form of Tridosha e.g., Vata (Ether+Air), Pitta (Fire), Kaph (Water+Earth). These three 'Doshas' are physiological entities in living beings.

In Ayurveda diagnosis included questioning, inference and examination of Pulse, Urine, Faeces, Tongue, Auditory and Tactile perceptions, Eyes and body as a whole. The treatment in Ayurveda system is individualized. Treatment in Ayurveda has two components Preventive and Curative.

Siddha System of Medicine

Siddha means achievements and Siddhars were saintly persons who achieved results in medicine. Siddha is largely therapeutic in nature.

The diagnosis of disease involves identifying its causes. Identification of causative factors is through the examination of pulse, urine, eyes, and study of voice, color of the body, tongue and the status of the digestive system.

The Siddha system is effective in treating chronic cases of liver, skin diseases especially "Psoriasis". The Siddha medicine which contains mercury, silver, arsenic, lead and sulphur have been found to be effective treating certain infectious diseases including venereal diseases.

Unani System of Medicine

The Unani System of Medicine is based on its well established knowledge and practices relating to promotion of positive health and prevention of diseases. It is very rich, time tested with its therapies having no side effects.

The Unani System emphasizes the use of naturally occurring, most herbal medicine and a few of animal, marine, and mineral origin. HK. Ajmal Khan has institutionalized the Ayurveda & Unani system of medicine and advocated the integration with science in understanding the Tibe-Unani and its application in treatment. He discovered Asrol (Rawolfia serpentina) which established its efficacy in the treatment of high blood pressure.

The treatment comprises of three components, namely preventive, promotive and curative. Unani system of Medicine is more efficacious in Rheumatic arthritis. Jaundice, Filariasis, Eczema, Sinusitis and Bronchial asthma etc.

Its efficacy in the treatment and management of cardiac diseases is being researched. Treatment is carried out in four forms i.e. Pharmacotherapy, Dietotherapy, Regimental Therapy and Surgery.

Homoeopathy

Homoeopathy is a specialized method of drug therapy of curing natural diseases by administration of drugs which has been experimentally proved to possess the power of producing similar artificial symptoms on healthy human beings.

Physicians from the time of Hippocrates (around 400 B.C.) observed that certain substances could produce symptoms that they were used to treat. However, it was a German doctor Dr. Christian Friedrich Samuel Hahnemann (1755-1843) who examined this observation more thoroughly and the emergence of Homoeopathy took place thereafter.

The first principle similar similibus curentur, states that a medicine, which can induce a set of symptoms in healthy human beings, would be capable of curing the similar set of symptoms in disease state. The second principle in single medicine at a time for a particular patient during the treatment on the basis of totality of symptoms. The third principle is minimum dose to be administered to a patient.

Medicines

In Homoeopathy, medicines are prepared from natural sources viz. Vegetable, Mineral, and Animal etc. There is no toxic or poisonous effect in the finished products of Homeopathic Medicines.

Strength of the system

Homoeopathy has its own areas of strength in therapeutics. Its curative capability extends to allergic manifestations, auto-immune disorders and viral

infections. Many Surgical, Gynecological & Obstetrical conditions, ailments affecting eyes, nose, ear, teeth, skin, sexual organs etc are curable through the homoeopathic treatment. Behavioral disorders, neurological problems, metabolic disorders successfully treated by the physicians of this system.

Homoeopathy has effective answer to addiction to drugs, tobacco and alcohol and is highly efficacious in ridding of addicts and their craving for these noxious substances.

Yoga and naturopathy

Yoga is primarily a way of life propounded by patanjali in a systemic form. It consists of eight components, namely, restraint, observance of austerity, physical postures, breathing exercise, restraining of sense organs, contemplation, meditation and Samadhi. These steps in the practice of yoga have potential for improvement of social and personal behaviour, improvement of physical health by encouraging better circulation of oxygenated blood in the body, restraining the sense organs and thereby inducing tranquility and serenity of mind.

Meditation can stabilize emotional changes and prevent abnormal functions of vital organs of the body. Studies have shown that meditation not only restrains the sense organs but also controls the nervous system.

Naturopathy is a system of medicine widely practiced, globally accepted and recognized by WHO. Naturopathy is a system of man building in harmony with constructive principles of Nature on physical, mental, moral and spiritual planes of living. It has great health promotive disease preventive and curative as well as restorative potential.

Amchi system of medicine

The Amchi system of Medicine is practiced in Ladakh district (J&K), Lahaul (Himachal Pradesh), Arunachal Pradesh, Sikkim and some regions of Himalayas.

The system traces its origin to Ayurvedic system of India. The medical system since its delivery by Lord Buddha, while meditating near Bodh Gaya, in course of time, had accumulated a huge literature and Amchi of great fame and repute were produced. Therapy under the system is divided into treatment by herbs, minerals, animal organs, spring and mineral water, moxibustion and spiritual powder.

The Central Council for Research in Ayurveda and Siddha under the Department of AYUSH is having an Amchi Research Unit at Leh to carry our research and to conduct survey of local drugs in Leh & Ladakh[3].

LEUCODERMA AND ITS MANAGEMENT

Give me a blue sky, brown mountain, silver falls and green around, nothing more I want is a popular saying. Nature is always wondered and admired at for its colorful beauty is being the main attraction.

But the whiteness offered by skin disorder-**Leucoderma** is often depressing for all the sufferers. Likewise in white skin people (even for leucoderma patient) hot sun UV rays penetrate more, putting them at risk for sunburn, skin cancer, etc.

Leucoderma is a miserable acquired skin disorder, making skin white due to loss of the melanin pigment. It is a non contagious disease**.** ***It is otherwise termed as Vitiligo***.

Incidences and risk personalities

Leucoderma is mostly restricted to the epidermis layer. It can occur in any age group (infants to old age), sex and races. Females are more commonly affected than males. The most commonly affected areas are face, neck, back, wrist, and hand, groin, gentials, and armpits etc. i.e. dark places, places where folds occur or where friction takes place.

Causes

Till now researchers have not identified any causative factor for leucoderma. It can also be familiar i.e. hereditary factor also has some role in the prevalence of leucoderma. Being emotionally upset may also precipitate or aggravate the complaint in certain circumstances. Some suspect skin, lack of sun exposure, infection, etc. to be the reason for the problem. All these risk factors only, but the real cause is still obscure or unknown.

The main causes of leucoderma are said to be excessive mental worry, chronic or acute gastric disorder, and impaired hepatic function such as jaundice, worms or other parasites in the alimentary canal, typhoid, a defective perspiratory mechanism, and burn injuries. Heredity is also a well-recognized causative factor.

Symptoms

Leucoderma appears as odd, harmless, white spots or patches disturbing the appearance of the sufferer. Mostly no other symptoms can be noted except whiteness of the skin. The whiteness usually starts as a small discolored white or pale or brown spot which spreads and becomes whiter day by day and becomes milky white in the course of time.

Prevention of spread

Check for any infective focus, worms, chemical, contact, etc if noted, they should be treated or eliminated start treatment early.

Do's

- Take medicines regularly in the early days itself so that complete cure can be achieved.
- Don't worry about leucoderma, since effective treatment can arrest the course of the disease and cure it.
- Use umbrella when out in the hot sun.

- Track all your eatables and habits to streamline all irritants or chemicals or sun exposure to arrest the spread of the disease.

Don'ts

- Avoid working or roaming in the sun.
- Avoid fast food, citrus fruits, coffee, tea, cold drinks, alcohol, beer, non-veg, egg ect, It can causes spread of the disease.
- Avoid multiple drugs or drug cocktails.
- Avoid chemical soaps

Pathologically:

A defect in enzyme Tyrosinase is held responsible for vitiligo. According to some Dermatologists, it is a Trophoneurosis and Melatonin, a substance secreted at nerve endings inhibits Tyrosinase, thus interfering in pigment formation.

Clinical Presentation of Vitiligo

Localized Type:

a) Focal - One or more macules in two single areas but not segmented.
b) Segmental - One or more macules in a dermatomal pattern.
c) Mucosal - Involvement of mucous membrane alone.

Generalised Type:

d) Acrofacial - Involvement of face and distal extremities.
e) Vulgaris - Scattered mascules in symmetrical or asymmetrical distribution.

Universalis

Total or nearly whole body involvement.

Who Is Affected by Vitiligo?

About 1 to 2 percent of the world's population, or 40 to 50 million people, have vitiligo. In the United States, 2 to 5 million people have the disorder. Ninety-five percent of people who have vitiligo develop it before their 40th birthday. The disorder affects all races and both sexes equally. Vitiligo seems to be more common in people with certain autoimmune diseases (diseases in which a person's immune system reacts against the body's own organs or tissues).

These autoimmune diseases include hyperthyroidism (an overactive thyroid gland), adrenocortical insufficiency (the adrenal gland does not produce enough of the hormone called corticosteroid), alopecia areata (patches of baldness), and pernicious anemia (a low level of red blood cells caused by failure of the body to absorb vitamin B12). Scientists do not know the reason for the association between vitiligo and these autoimmune diseases. However, most people with vitiligo have no other autoimmune disease. Vitiligo may also be hereditary, that is, it can run in families. Children whose parents have the disorder are more likely to develop vitiligo. However, most children will not get vitiligo even if a parent has it, and most people with vitiligo do not have a family history of the disorder.

How Is Vitiligo Diagnosed?

If a doctor suspects that a person has vitiligo, he or she usually begins by asking the person about his or her medical history. Important factors in a person's medical history are a family history of vitiligo; a rash, sunburn, or other skin trauma at the site of vitiligo 2 to 3 months before depigmentation started; stress or physical illness; and premature graying of the hair (before age 35). In addition, the doctor will need to know whether the patient or anyone in the patient's family has had any autoimmune diseases and whether the patient is very sensitive to the sun. The doctor will then examine the patient to rule out other medical problems.

The doctor may take a small sample (biopsy) of the affected skin. He or she may also take a blood sample to check the blood-cell count and thyroid function. For some patients, the doctor may recommend an eye examination to check for uveitis (inflammation of part of the eye). A blood test to look for the presence of antinuclear antibodies (a type of autoantibody) may also be done. This test helps to determine if the patient has another autoimmune disease.

General Treatment

There is no scheduled, conservative treatment at all with rudimentary knowledge, everyone speaks more, but none of the internal or external claims guarantee for solid cure. No single therapy for leucoderma produces good result in all patients.

What Treatment Options Are Available?

The goal of treating vitiligo is to restore the function of the skin and to improve the patient's appearance. Therapy for vitiligo takes a long time. It usually must be continued for 6 to 18 months. The choice of therapy depends on the number of white patches and how widespread they are and on the patient's preference for treatment. Each patient responds differently to therapy and a particular treatment may not work in everyone.

TREATMENT OPTIONS FOR VITILIGO

Medical Treatments

Topical steroid therapy

- Topical Psoralen photo chemotherapy
- Oral Psoralen photo chemotherapy
- Depigmentation

Surgical Therapies

- Autologous skin grafts
- Skin grafts using blisters
- Micropigmentation (tattooing) and Autologous melanocyte transplants.

Adjunctive Therapies

- Sunscreens
- Cosmetics

Counseling and support

Current treatment options for vitiligo include medical, surgical, and adjunctive therapies (therapies that can be used along with surgical or medical treatments).

MEDICAL TREATMENTS

TOPICAL STEROID THERAPY

Steroids may be helpful in repigmenting (returning the color to white patches) the skin, particularly if started early in the disease. Corticosteroids are a group of drugs similar to the hormones produced by the adrenal glands (such as cortisone). Doctors often prescribe a mild topical corticosteroid cream for children under 10 years old and a stronger one for adults. Patients must apply the cream to the white patches on their skin for at least 3 months before seeing any results. It is the simplest and safest treatment but not as effective as Psoralen photochemotherapy. The doctor will closely monitor the patient for side effects such as skin shrinkage and skin striae (streaks or lines on the skin).

Psoralen Photochemotherapy

Psoralen photochemotherapy (Psoralen and ultraviolet A therapy, or PUVA) is probably the most beneficial treatment for vitiligo available in the United States.

However, it is time-consuming and care must be taken to avoid side effects, which can sometimes be severe. Psoralens are drugs that contain chemicals that react with ultraviolet light to cause darkening of the skin. The treatment involves taking Psoralen by mouth (orally) or applying it to the skin (topically). This is followed by carefully timed exposure to ultraviolet A (UVA) light from a special lamp or to sunlight. Patients usually receive treatments in their doctor's offices so that they can be carefully watched for any side effects. Patients must minimize exposure to sunlight at other times. The goal of PUVA therapy is to repigment the white patches.

Topical Psoralen Photochemotherapy

Topical Psoralen photochemotherapy often is used for people with a small number of depigmented patches (affecting less than 20 percent of the body). It is also used for children 2 years old and over who have localized patches of vitiligo. Treatments are done in a doctor's office under artificial UVA light once or twice a week. The doctor or nurse applies a thin coat of Psoralen to the patient's depigmented patches about 30 minutes before UVA light exposure. The patient is then exposed to an amount of UVA light that turns the affected area pink. The doctor usually increases the dose of UVA light slowly over many weeks. Eventually, the pink areas fade and a more normal skin color appears.

After each treatment, the patient washes his or her skin with soap and water and applies a sunscreen before leaving the doctor's office.

There are two major potential side effects of topical PUVA therapy:

(1) Severe sunburn and blistering and (2) too much repigmentation or darkening of the treated patches or the normal skin surrounding the vitiligo (hyperpigmentation). Patients can minimize their chances of sunburn if they avoid exposure to direct sunlight after each treatment. Hyperpigmentation is usually a temporary problem and eventually disappears when treatment is stopped.

Oral Psoralen Photohemotherapy

Oral PUVA therapy is used for people with more extensive vitiligo (affecting more than 20 percent of the body) or for people who do not respond to topical PUVA therapy**. Oral Psoralen is not recommended for children under 10 years of age because of an increased risk of damage to the eyes, such as cataracts.** For oral PUVA therapy, the patient takes a prescribed dose of Psoralen by mouth about 2 hours before exposure to artificial UVA light or sunlight. The doctor adjusts the dose of light until the skin areas being treated become pink. Treatments are usually given two or three times a week, but never on two days in a row. For patients who cannot go to a PUVA facility Psoralen may be used with natural sunlight exposure, with careful instruction and frequent monitoring by the treating physician.

Known side effects of oral Psoralen include sunburn, nausea and vomiting, itching, abnormal hair growth, and hyperpigmentation. **Oral Psoralen photochemotherapy may increase the risk of skin cancer.** To avoid sunburn and reduce the risk of skin cancer, patients undergoing oral PUVA therapy should apply sunscreen and avoid direct sunlight for 24 to 48 hours after each treatment. Patients should also wear protective UVA sunglasses for 18 to 24 hours after each treatment to avoid eye damage, particularly cataracts.

Depigmentation

Depigmentation involves fading the rest of the skin on the body to match the already white areas.

For people who have vitiligo on more than 50 percent of their body, depigmentation may be the best treatment option. Patients apply the drug monobenzylether of hydroquinone (monobenzone or Benoquin) twice a day to pigmented areas until they match the already depigmented areas. Patients must avoid direct skin-to-skin contact with other people for at least 2 hours after applying the drug.

The major side effect of depigmentation therapy is **inflammation (redness and swelling) of the skin. Patients may experience itching, dry skin or abnormal darkening of the membrane that covers the white of the eye.** Depigmentation is permanent and cannot be reversed. In addition, a person who undergoes depigmentation will always be abnormally sensitive to sunlight.

SURGICAL THERAPY

All surgical therapies must be viewed as **experimental because their effectiveness and side effects remain to be fully defined.**

Autologous Skin Grafts

In autologous (use of a person's own tissues) skin graft, the doctors removes skin from one area of a patient's body and attaches it to another area. This type of skin grafting is sometimes used for patients with small patches of vitiligo. The doctor removes sections of the normal, pigmented skin (donor sites) and places them on the depigmented areas (recipient sites). There are several possible complications of autologous skin grafting. **Infections may occur at the donor or recipient sites.**

The recipient and donor sites may develop scarring, a cobblestone appearance or a spotty pigmentation, or may fail to repigment at all. Treatment with grafting takes time and is costly, and most people find it neither acceptable nor affordable.

Skin Grafts Using Blisters

In this procedure, the doctor creates blisters on the patient's pigmented skin by using heat, suction, or freezing cold. The tops of the blisters are then cut out and transplanted to a depigmented skin area.

The risks of blister grafting include **the development of a cobblestone appearance, scarring, and lack of repigmentation**. However, there is less risk of scarring with this procedure than with other types of grafting.

Micropigmentation (Tattooing)

Tattooing implants pigment into the skin with a special surgical instrument. This procedure works best for the lip area, particularly in people with dark skin; however, it is difficult for the doctor to match perfectly the color of the skin of the surrounding area. **Tattooing tends to fade over time. In addition, tattooing of the lips may lead to episodes of blister outbreaks caused by the Herpes Simplex Virus.**

Autologous Melanocyte Transplants

In this procedure, the doctor takes a sample of the patient's normally pigmented skin and places it in a laboratory dish containing a special cell culture solution to grow melanocytes.

When the melanocytes in the culture solution have multiplied, the doctor transplants them to the patient's depigmented skin patches. This procedure is currently experimental and is **impractical for the routine care of people with vitiligo.**

ADJUNCTIVE THERAPY

Sunscreens

People, who have vitiligo, particularly those with fair skin, should use a sunscreen that provides protection from both the UVA and UVB forms of ultraviolet light. Sunscreen helps protect the skin from sunburn and long-term damage. Sunscreen also minimizes tanning, which makes the contrast between normal and depigmented skin less noticeable.

Cosmetics

Some patients with vitiligo camouflage depigmented patches with stains, makeup, or self-tanning lotions. These cosmetic products can be particularly effective for people whose vitiligo is limited on exposed areas of the body. Dermablend, Lydia O'Leary, Clinique, Fashion Flair, Vitadye, and Chromelin offer makeup or dyes that patients may find helpful for covering up depigmented patches.

Tab 1 Medicinal plants used in Leucoderma[4].

1	Abrus precatorius Linn	2	Acacia catechu Willd
3	Acacia foprnesiana Willd	4	Acacia ferruginea DC
5	Acacia nilotica Del	6	Aconitum chasmanthum Stapf
7	Aconitum falconeri Stapf	8	Aconitum rugata Lam
9	Acorus calamus Linn	10	Adhatoda vasica Nees
11	Aglaia odoratissima Blume	12	Albizia amara Boivin
13	Albizia lebbek Benth	14	Allium cepa Linn
15	Allium sativum Linn	16	Alpinea galanga Willd
17	Alpinia khulanjan Sheriff	18	Alpinia officinarum Hance
19	Alstonia scholaris R Br	20	Altingea excelasa Noronha
21	Anararabtgus tristis Linn	22	Ammi majus linn
23	Anacardium occidentale Linn	24	Anacyclus pyrethrum DC
25	Amemone obustifolia D Don	26	Anethum sowa Roxb
27	Anthriscus cerifolium Hoffm	28	Apium graveolen Linn
29	Aquillaria agallocha Roxb	30	Argemone mexicana Linn
31	Aristolchia indica Linn	32	Artemisia siversiana Willd
33	Astragalus hamosus Linn	34	Atropa belladomma Linn
35	Azadirachta indica A juss	36	Bacopa montanum Linn

37	Balanites aegyptica Del	38	Baleopermum montanum Muell-Arg
39	Bambusa arundinaeca Willd	4o	Barleria priotis Linn
41	Barleria strigosa Willd	42	Bauhimea variegate Linn
43	Blepharis edulis pers	44	Boswelia serrata Roxb
45	Brassica campestris Linn	46	Brassica nifra Koch
47	Butea monosperma (Lam) Kuntz	48	Calamus rotang Linn
49	Calotroppis gigantean R Br	50	Canscora decussate Roem et Sch
51	Capparis sepiaria Linn	52	Careya arborea Roxb
53	Carthamus tinctorius Linn	54	Carum carvi Linn
55	Casearia esculenta Roxb	56	Cassia absus Linn
57	Cassia angustifolia vahl	58	Cassia tora Linn
59	Cedrus deodara (Roxb) Lour	60	Celastrus paniculata Willd
61	Centella asiatica Urban	62	Centipeda minima Br et Asch
63	Centratheryum anthelminitcum	64	Cephalandra indica Naud
65	Chenopodium album Linn	66	Cinnamomum zeylanicum Blume
67	Cissus adanta Roxb	68	Cissus setosa Roxb
69	Citrullus colocynthis Schrad	70	Cleome brachycarpa Vahl
71	Cleome chelidonni Linn	72	Cleome icosandra Linn
73	Clerodendron infortuatum Linn	74	Clitorea ternatea Linn
75	Commiphora agalocha Engl	76	Commiphora mukul Hook ex Stocks
77	Convolvulus scammonia Linn	78	Coptis teeta wall
79	Coriandrum sativum Linn	80	Corydalis govniana wall
81	Croton tiglium Linn	82	Cuminum cyminum Linn
83	Curcuma angustifolia Roxb	84	Curcuma aromatica Salisb
85	Curcuma caesia Roxb	86	Curcuma longa Linn
87	Curcuma zedoaria Rosc	88	Dalbergia sissoo Roxb
89	Datura metel Linn	90	Datura stramonium Linn
91	Delphinium denudatum Wall	92	Dioscorea bulbifera Linn
93	Dolichos biflorus Linn	94	Dolichos falcatus Klun
95	Drogea volubilis Benth	96	Ecballium elaterium ARich
97	Eclipta alba Massk	98	Eclipta erecta Lamk
99	Embelia ribes Burm f	100	Emblica officinalis Gaerth
101	Enhydra fluctuans Lour	102	Eruca sativa Mill
103	Euporbia meriifolia Linn	104	Evolvulus alsinoldes Linn
105	Fagonia cretica linn	106	Feronia limomia Swingl
107	Ficus carica Linn	108	Ficus hispida Linn
109	Ficus micrlcarpa E Vill	110	Ficus racemosa Linn
111	Ficus religiosa Linn	112	Ficus tsiela Roxb
113	Fumaria indica pugsley	114	Geranium wallichianum Sweet
115	Girardinia heteraphylla DC	116	Gisekia pharnaceildes Linn
117	Glorisa superba Linn	118	Gymnema sylevestre R Br
119	Gynocardia ldlrata R Br	120	Haemidesmus indicus R Br

121	Heracleum candicans Wall	122	Heracleum canescens Lind
123	Heracieum pinnatumWild	124	Holarrhena antidysentrica Wall
125	Holostemma annulare K Schum	126	Hydnocarpus Kurzii Warb
127	Hyoscymus niger Linn	128	Indigofera tinctoria Linn
129	Ipomea hederacea Jacq	130	Ipomea reninflrmis Choisy
131	Ipomea raptans Plir	132	Iris versiclolr Linn
133	Lactuca scariola Linn	134	Lamprachemium microcephalum
135	Lawsonia inermis Linn	136	Lepidium sativum Linn
137	Lolium femulentum Linn	138	Luffa acutangula Roxb
139	Lupinus albus linn	140	Macrotomea benthame DC
141	Macrotomea perennis Bois	142	Martynia annua Linn
143	Melia azardirach Bois	144	Melia acadirachta Linn
145	Meliotus alba Desr	146	Meliotus officinalis Desr
147	Mentha sylvestris Linn	148	Mimosa pudica Linn
149	Moringa concanensis Nimmo	150	Mofinga oleifera Lam
151	Murraya koenigii Spreng	152	Musa paradisiacal Linn
153	Mussaenda glabrata Hutich	154	Nardostachys jatamansi DC
155	Nelumbo mucifera Gaertn	156	Nerium odorum Soland
157	Micotians rustica Linn	158	Nicotianatabacum Linn
159	Nigella sativa Linn	160	Nymphaea stellata Willd
161	Ocimum basilicum Linn	162	Ocimum samctum Linn
163	Onosma bracteatum Will	164	Onosma echilides Linn
165	Operculiana turpethum satiba Linn	166	Opuntia dillunii Haw
167	Origanum majorana Linn	168	Oroxylon indicum Vent
169	Orthosiphon pallidus Royle	170	Ougeinia oojeinensis Hochr
171	Oxystelma esculenta R Br	172	Paederia foetida Linn
173	Pandanus tectorius Sol	174	Paris polyphylla Smith
175	Pentatropis cynachoides R Br	176	Phaseolus radiatus Lour
177	Picrorrhiza kurroa Royle ex Behth	178	Pinus sylvestris Linn
179	Piper longum Linn	180	Plantanus orientalis Linn
181	Plumbago indica Linn	182	Plumbago zeylanica Linn
18	Pongamia pinnata Merr	184	Prospis juliflora DC

3			
185	Prunus amygdalus Stok	186	Prunus cerasoidea D Don
187	Prunus perrica Batsch	188	Psoralea corylifolia Linn
189	Pterocarpus marsupium Roxb	190	Punica granatum Linn
191	Randia dumetorum Lam	192	Ranunculus scleratus Linn
193	Raphanus sativus Linn	194	Rheum emodi Wall
195	Ricinus communis Linn	196	Rubia cordifolia Linn
197	Rumex besicarium Linn	198	Sapindus mukorossi Gaerth
199	Sasbania sesban Merr	200	Ssussurea lappa Clarke
201	Semecarpus anacardium Linn	202	Solanum indicum Linn
203	Solanum melongena Linn	204	Solanum nigrum Linn
205	Sphaeranthus indicus Linn	206	Spinacea oleracea Linn
207	Strychmos nux vomica Linn	208	Swertia chirata Buch Ham
209	Tamarix articulate Vahl	210	Tamarix indica Linn
211	Tamarix troupii Hole	212	Tecomelia undulate Seem
213	Tectona grandis Linn	214	Terminalia arjuna Wright &Arn
215	Terminalia belerica Rlxb	216	Terminalia chebula Retz
217	Terminalia citrine roxb	218	Thevetia neriifolia Juss
219	Tricholepis glaberrima DC	220	Trichosanthes cucurmeriana Linn

221	Trigonella foenumgraecum Linn	222	Urginea indica Kunth
223	Urtica dioica Linn	224	Uritica parviflora Rlxb
225	Vicia faba Linn	226	Viola serpens Wall
227	Viola tricolor Linn	228	Vilex megundo Linn
229	Vitex triflioa Linn	230	Wedelia calendulacea Less
231	Widhania somnifera Dunal	232	Wrightia tinctoria R Br
233	Xanthium stumarium Linn	234	Zanthoxylum acanthopodium DC
235	Zanthoxulum alatum Roxb	236	Zanthoxylum oxyphyllum Edgew
237	Zingiber officinale Rosc		

MICROSPONGE DRUG DELIVERY SYSTEM (MDS)

During the few years, in pharmaceutical research much attention has been given to the controlled release of Topical drugs. By the use of this approach, controlling the therapeutic efficacy of these drugs, reduction in the total dose needed and a reduction in the side effects can be provided. [5] For this purpose, Microsponges are one of the systems which are in trial for controlled drug delivery. Microsponges are porous, polymeric microspheres that are used mostly for topical and recently for oral administration. Microsponges are microscopic spheres capable of absorbing skin secretions, therefore reducing oiliness and shine from the skin. Spherical particles composed of clusters of even tinier spheres are capable of holding four times their weight in skin secretions. The particle size ranges between 5-300 micrometer[6].

The most closely related systems are Microcapsules and Microspheres. Microcapsules are spherical particles containing an active agent in the core, surrounded by a polymeric membrane. Microspheres are spherical particles containing the active agent dispersed in a polymeric matrix.

The major distinguishing feature between Microsponge and Polytrap systems and Microcapsules, or Microspheres, is structure of Microsponge and Polytrap systems are **highly porous**, while Microspheres or Microcapsules are solid particles with *no internal voids.*

A variety of active agents can be entrapped in a single Microsponge system and to release them at desired rates. The Microsponge system **can prevent excessive accumulation of ingredients within the epidermis and the dermis.** Potentially, the Microsponge system can reduce significantly the irritation of effective drugs without reducing their efficacy. Further these porous microspheres with active ingredients can be incorporated in to formulations such as creams, lotions and powders.

Microsponges consisting of non-collapsible structures with porous surface through active ingredients are released in a controlled manner. This delivery system can be incorporated into conventional dosage forms such as creams, lotions, **gels**, ointments, and powder and share a broad package of benefits.

Advantages of MDS [7]

- Advance oil control – absorbs up to 6 times its weight without drying.
- Extended release – continuous action up to 12 hours.
- Reduced irritation – better tolerance means broader consumer acceptance.
- Improve product aesthetics – gives product an elegant feel.
- Improve the stability – thermal, physical and chemical.
- Allow incorporation of immiscible.
- Allow for novel product form.
- Improve the material processing.
- Conversion of indigenous drugs in to Novel drug delivery system

Method of preparation

Polymerization

The porous microspheres are prepared by Suspension Polymerization method in liquid-liquid systems. [12], In this preparation, the monomers are first dissolved along with active ingredients in a suitable solvent solution of monomer and are then dispersed in the aqueous phase, which consist of additives (surfactant, suspending agents, etc. to aid in formation of suspension).

The polymerization is then initiated by adding catalyst or by increasing temperature or irradiation. The various steps in the preparation of Microsponge are summarized as

- Selection of monomer or combination of monomers
- Formation of chain monomers as polymerization begins
- Formation of ladders as a result of cross linking between chain monomers
- Folding of monomer ladder to form spherical particles
- Agglomeration of Microspheres which give rise to formation of bunches of Microspheres
- Binding of bunches to form Microsponges

The polymerization process leads to the formation of a reservoir type of system, which opens at the surface through pores. In some cases an inert liquid immiscible with water but completely miscible with monomer is used during the polymerization to form the pore network. After the polymerization the liquid is removed leaving the porous microspheres, i.e., Microsponges. Impregnating them within preformed Microsponges then incorporates the functional substances. Some times solvent may be used for faster and efficient incorporation of the active substances. The Microsponges act as a topical carriers for variety of functional substances, e.g. anti acne, anti inflammatory, anti purities, anti fungal, rubefacients, etc[6].

Quasi – Emulsion solvent diffusion method

Microsponges prepared by a quasi-emulsion solvent diffusion method using an external phase of containing distilled water and polyvinyl alcohol (PVA) 72 000. The internal phase consisted of drug, ethyl alcohol, polymer and triethylcitrate (TEC)[8] , Which is added at an amount of 20% of the polymer in order to facilitate the plasticity[9]. At first, the internal phase was prepared at 60°C and added to the external phase at room temperature. After emulsification, the mixture is continuously stirred for 2 hours. Then the mixture is filtered to separate the Microsponges. The product is washed and dried by vacuum oven at 40°C for 24 hours.

Variables during in the preparation [10]

- Drug and Polymer ratio
- Effect of stirring time
- Effect of stirring speed
- Effect of composition of internal and external phase
- Viscosity

POLYMERS FOR DRUG DELIVERY

Role of polymers [11]

The polymer can protect the drug from the physiological environment and hence improve its stability in vivo. By altering the polymer structure in certain ways the rate at which the drug release can be controlled. Design the polymers that are more hydrophobic will cause the drug to be released slowly, while a polymer that is less hydrophobic will release its drug faster.

Characteristic of ideal polymer [12]

1. It should be chemically inert and free from leachable impurities.
2. It should have good mechanical strength.
3. It should be easy and inexpensive to fabricate.
4. It should be easily sterilized.
5. It should demonstrate acceptable shelf life.

Drug release mechanism [13]

Two broad categories of polymer system have been studied. The reservoir device involves the encapsulation of a drug with in the polymer shell, while the matrix device describes a system in which a drug is physically entrapped with in a polymer net work. The drug will be released over time either by diffusion out of the polymer matrix or by erosion (due to degradation) of the polymer or by a combination of two mechanisms.

Polymers have been classified broadly as [13]

- ❖ Natural polymers - Albumin, Starch, Dextran, Gelatin Fibrinogen, Chitosan ect.
- ❖ Synthetic polymers - Polymethyl methacrylate, Polymethyl methacrylate, Copolymers: Polymethyl Cyanoacrylate, Polyacrylamide, Poly acryl starch, Poly lactic acid ect.

Polymers are further classified on the basis of their interaction with water.

Non-biodegradable polymers

They are inert in the environment of use and eliminated intact from the site of administration and is the rate limiting factor.

Eg: Polyethlene Vinyl Acetate (PVA). Poly Dimethl Siloxane (PDS), Poly Urethane (PU), Ethyl Cellulose (EC), Cellulose Acetate (CA) etc.

Hydro Gels

These type of polymers swell but do not dissolve when brought into contact with water. They are inert and remover intact from the site of administration.

Eg: Poly Hydroxyl Ethyl Metha Acrylate (PHEMA), cross linked Poly Vinyl Alcohol (PVA), cross linked Poly Vinyl Pyrrolidone (PVP), Polyacrylamide, Dextran.

Soluble polymers

These are moderate weight uncross-linked polymers that, dissolve in water.

Eg: Poly Ethylene Glycol (PEG), Hydroxy Propyl Methyl Cellulose, Methocel and copolymers of methamers of methacrylic acid, Acrylic methyl acid methyl ester (Eudragit L).

Biodegradable polymers

These slowly disappear from the site of administration in to a chemical reaction like hydrolysis.

Eg: Poly Lactic Acid (PLA), Poly Glycolic Acid (PGA), Poly Capro Lactone (PCL) [12].

Polymers used in MDS

- Polymethacrylate
- Ethyl cellulose

Evaluation of MDS (Microsponge Drug delivery System) [14 – 16]

Prepared Microsponges are generally analyzed by following parameters

- Polymer composition
- Particle size
- Surface topography
- Pore diameter
- Drug content
- Loading efficacy
- Product yield

- Drug release studies

Drugs explored in Microsponge delivery [7]

- Ketoprofen
- Benzyl peroxide
- Retinol
- Fluconazole
- Ibuprofen
- Tretinoin
- Trolamine

MICROSPONGE INCORPORATED IN TO TOPICAL DRUG DELIVERY

Topical Drug Delivery

Topical semi – solids are preparations designed to exert local activity when applied to the skin or mucous membranes. The main medicinal applications of semi – solids are as protective, emollient, and **therapeutic agents**. They include such dosage forms as creams, **gel**, ointments, pastes and poultices[17].

Tab 2 Commercial Microsponge Formulations [7]

GELS NAME	**DRUG**	**REMARKS/USE**
Afirm 3X	Retinol	Design to treat photodamaged skin
EpiQuin[TM]	Vitamin A	Gradual treatment of ultraviolet induced dyschromia and discoloration
RnUn-A Micro	Tretinoin	For the treatment of acne vulgaris
Curan[R]	5-flourouracil	For actinic keratois
MicroPeel[R]	Salicylic acid	Freeing the skin of all dead cells and stimulating the skin renewal process.
Sprtscream[R] XS	Trolamine	For the temporary relief of pain associated with musculoskeletal soreness and discomfort

Gels are transparent or translucent semi – solid or solid preparation consisting of solutions or dispersions of one or more active ingredients in suitable hydrophilic or hydrophobic bases. They are made with the aid of gelling agent. Usually gels exhibit pseudoplastic flow properties and those made with synthetic or semi – synthetic polymers with a high degree of cross – linking have relative high yield value and low viscosity.

Product tends to be smooth, elegant, and produce cooling effect because of evaporation of water. They may also dry out to form films. Films adhere well to the skin and are usually easily removed by washing.

Gels (sometimes called Jellies) are semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Where the gel mass consists of a network of small discrete particles, the gel is classified as a **two-phase system** (e.g., Aluminum Hydroxide Gel). In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes referred to as a magma (e.g., Bentonite Magma).

Both gels and magmas may be thixotropic, forming semisolids on standing and becoming liquid on agitation. They should be shaken before use to ensure homogeneity and should be labeled to that effect.

Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Single-phase gels may be made from synthetic macromolecules (e.g., **Carbomer**) or from natural gums (e.g., Tragacanth).

The latter preparations are also called mucilages. Although these gels are commonly aqueous, alcohols and oils may be used as the continuous phase.

For example, mineral oil can be combined with a polyethylene resin to form an oleaginous ointment base. Gels can be used to administer drugs topically or into body cavities (e.g., Phenylephrine Hydrochloride Nasal Jelly)[18].

SKIN STRUCTURE

The skin is one of the most extensive organs of the human body covering an area of about $2m^2$ in an average human adult. This multilayered organ receives approximately one – third of all blood circulating through the body.

The integument is composed of skin and its associated tissues sweat glands, sebaceous glands, hair, and nails. It is largest organ of the body (6% of the body weight).Covers the entire body continuous with the digestive system (lips and anus), respiratory system (nose), and urogenital system (urethra).

SKIN FUNCTION

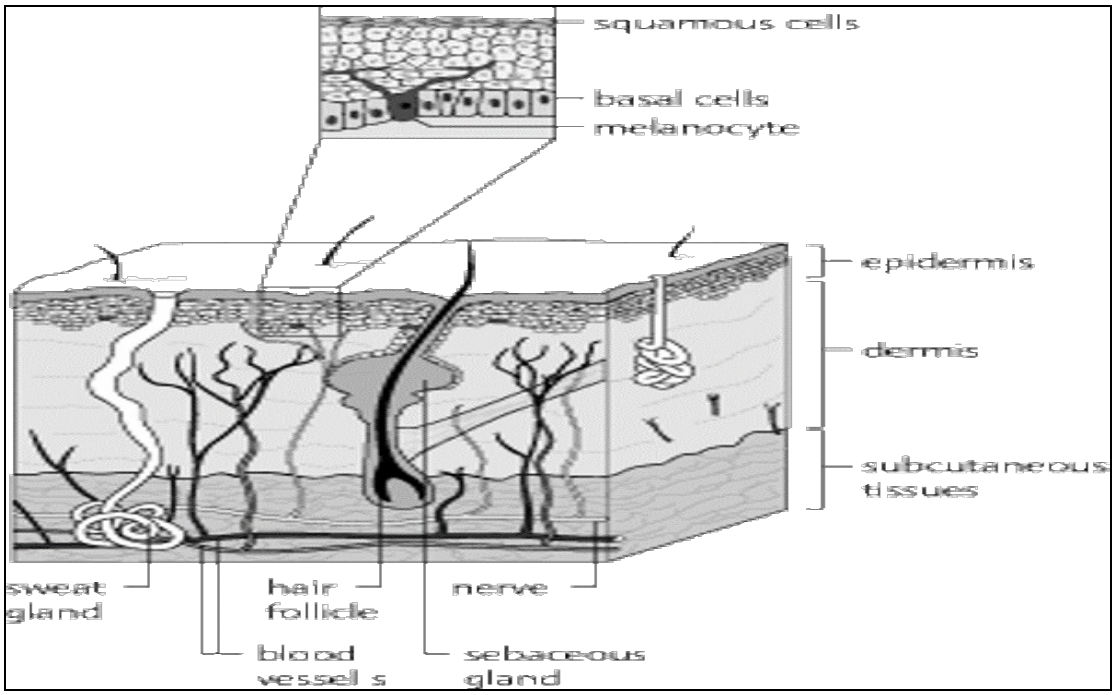
1) Serves as a barrier to physical, biological, and chemical agents and to radiation
2) Prevents desiccation
3) Regulates body temperature (thermoregulation) by evaporative cooling (perspiration), heat radiation at the surface of the body (blood circulation), and serves as insulation (a minor function in humans)
4) Site of excretion through sweat glands
5) Photochemical production of vitamin D
6) Serves as a sensory organ

Skin is composed of two primary layers

The epidermis is the ectodermally derived outer layer composed of keratinized stratified squamous epithelium.

The dermis is the mesodermally derived layer of dense irregular collagenous connective tissue that underlies and interdigitates with the epidermis. The hypodermis is a loose connective tissue containing varying amounts of adipose that underlies and supports the skin, it is the superficial fascia that covers the entire body.

Fig 1 Structure of Skin



THE EPIDERMIS:

The epidermis is an epithelium comprised of keratinocytes undergoing a program of sequential differentiation. The four major layers arise from the sequential differentiation of cells migrating from the basal layer to the surface.

The basal layer (stratum basale) is the deepest layer; it sits on the dermis. This layer is comprised of a single layer of mitotically active cuboidal or columnar epithelia. This is a major region of mitotic activity. Roughly half of the basal cell keratinocytes are mitotically active. The others become active after wounding. Hyperproliferative diseases like psoriasis result from an inbalance of stimulatory and inhibitory growth factors acting on these cells. Basal cells are attached to each other by desmosomes that contain cadherins. Basal cell layers attach to the basement membrane (basal lamina) on which they reside via hemidesmosomes that contain integrins.

Desmosomes and hemidesmosomes anchor intermediate filaments, which are comprised of keratins in epithelial cells, to the cell surface. Two types of keratins, type I keratins, which are acidic and type II keratins, which are basic, are required to form an intermediate filament. In epidermis, different pairs of keratin genes are expressed as the cells migrate upward from the basal layer and proceed through their differentiation program. For example, keratin 5 and 14 are expressed in the basal layer, while keratins 1 and 10 are found in the spinous layer.

The spinous layer (stratum spinosum) is the thickest layer of epidermis. The cells in this layer arise from the migration from the basal layer and lose their adhesion to the basement membrane and adhere to other keratinocytes. Some cells in this layer are also mitotically active. The cells in this layer attain a more flattened shape and have increased amounts of keratin containing intermediate filaments and desmosomes.

The granular layer (stratum granulosum) is characterized by the presence of keratohyalin granules among the keratin filaments; it consists of 3-5 layers of flattened keratinocytes. Keratohyalin granules contain the protein filaggrin, which serves to bundle the keratin filaments together. This is the most superficial layer of the epidermis that still has nuclei.

These layers (and the upper spinous layers, to a lesser degree) contain membrane coating granules (lamellar granules).

The cornified layer (stratum corneum) is the most superficial layer of skin and is composed of layers of dead cells. There is a sharp transition between the granule layer and cornified layer. Keratin filaments are highly crosslinked by the formation of S-S bonds and the bundling activity of keratohyalin. Membrane coating granules are exocytosed into the intracellular space. The high lipid content forms a permeability barrier for water retention and resistence. This layer provides 98% of the water retention ability of the epidermis. The plasma membrane becomes thick, due to the deposition and crosslinking (via transglutaminase, an enzyme that

covalently links glutamine to lysine) of proteins, like involucrin, along its inner surface to form the cornified envelope.

These cells lack nuclei and other organelles but have numerous keratin filaments. Cells farther from the skin surface have desmosomes, while those nearer to the surface do not and will be sloughed. Epidermis turns over about every 14-30 days depending on the region of skin. Different areas of skin show differing thickness of epidermis.

1. The epidermis thickens in response to use (abrasion).
2. The dryness of skin serves as an inhospitable environment for growth of microorganisms.

THE DERMIS:

The **dermis** is a dense, irregular, mesodermally derived, connective tissue, composed of collagen (mostly type I), elastin, and glycosaminoglycans. It is much thicker than the epidermis, comprising 80-90% of the total dermis and epidermis.

It contains extensive vasculature, neurons, smooth muscle, and fibroblasts. It is the principal mechanical barrier of skin. Its networks of elastic fibers function to support the epidermis and bind the skin to the deeper hypodermis. The dermis contains two layers, the **papillary layer** and the **reticular layer**. The papillary layer is a loosely woven, superficial connective tissue region that interdigitates with the epidermal ridges and the deeper reticular layer.

A key feature of this layer is the dermal ridges (dermal papillae) that extend up in ridges into the overlying epidermis and interdigitate with epidermal invaginations (epidermal ridges). The dermal ridges contain **Meissner's corpuscles**, encapsulated nerve endings, and capillary loops that provide nutrients to the avascular epidermis. A basal lamina supports the basal layer of the epidermis. Thin collagenous and elastic fibers underlie the basal lamina. Fibroblasts, mast cells, and

macrophages occupy the papillary layer. The reticular layer resides below the papillary layer.

It is comprised of coarse collagenous and elastic fibers (irregular dense connective tissue) and relatively few cells.Arteries and veins run through the hypodermis and branch upward to form plexuses of anastomosing vessels. The cutaneous plexus resides at the junction of the hypodermis and dermis, and the papillary plexus resides just beneath the epidermis. This system provides nourishment to the dermis and by diffusion to the epidermis, which is avascular. The vascular system functions in thermoregulation. Blood flow is controlled by contraction of arterioles and venules to send blood through the capillary bed for heat radiation. In some regions of skin, arteriovenous anastomoses, or shunts, can send blood directly from the arterioles to venules in order to reduce heat loss.

The dermis contains neuronal elements for touch, pain, itch, and temperature reception.

1. Some receptors are free nerve endings.
2. Other nerve endings associate with Merkel cells in the epidermis.
3. **Meissner's corpuscles** reside in the dermal papillae and function as mechanoreceptors in touch perception.
4. **Pacinian corpuscles** are found deep in the dermis (and in the hypodermis) and function in pressure sensation[19].

LITERATURE SURVEYED

LITERATURE SERVEYED OF MICROSPONGE

- **Çomoğlu et.al**[5] Reported the Effect of Different Polymers on Microsponge Formation. By the use of this approach, controlling the therapeutic efficacy of these drugs, reduction in the total dose needed and a reduction in the side effects can be provided. The Microsponges were prepared by emulsion-solvent diffusion method using different polymers such as ethyl cellulose, Eudragit RS 100 and Eudragit RS 100 and ethyl cellulose mixtures at different ratios.

- **Patel Geeta et.al**[7] Reported Use of a Microsponge in drug delivery systems. The Microsponge delivery system is a unique technology for the controlled release of topical agents and consists of macroporous beads, typically 10-25 microns in diameter, loaded with active agent. When applied to the skin, the Microsponge releases its active ingredient on a time mode and also in response to other stimuli (rubbing, temperature, pH, etc). Microsponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, improved stability, increased elegance, and enhanced formulation flexibility.

- **Jelvehgari et.al.**[10] Carried out preparation, characterization and release studies of Benzoyl peroxide (BPO) Microsponge drug delivery prepared by quasia emulsion solvent diffusion method. The aim of the present study was to produce ethylcellulose microparticles containing BPO which were able to control the release of BPO to the skin and reduces the side effect of commercial BPO such as irritation and percutaneous absorption. Generally, an increase in the ratio of drug: polymer resulted in a reduction in the release rate of BPO from Microsponges.

- **Mine Orlu et.al**[20] Prepared a Novel colon specific drug delivery system containing flurbiprofen (FLB) Microsponges. The colon specific formulations

were prepared by compression coating and also pore plugging of Microsponges with pectin: hydroxypropylmethyl cellulose (HPMC) mixture followed by tabletting.

- **Comoglu et.al**[21] Carried out the enhancement of ketoprofen bioavailability by formation of microsponge tablets. An in vivo study was designed to evaluate the pharmacokinetic parameters and to compare them with the commercially available ketoprofen retard tablets containing the same amount of the active drug. The new modified release tablets showed a slower absorption rate and peak levels were reached 8 h after administration.

- **Tansel Çomoglu et.al**[22] Reported the effects of pressure and direct compression on tabletting of Microsponges. Microsponge delivery is used both oral and topical purpose. Ketoprofen was used as a model drug for systemic drug delivery of Microsponges. Microsponges were prepared by quasi-emulsion solvent diffusion method with Eudragit RS 100 and afterwards tablets of Microsponges were prepared by direct compression method. Different pressure values were applied to the tablet powder mass in order to determine the optimum pressure value for compression of the tablets.

- **Nokhodchi et.al**[23] Reported Factors affecting the morphology of benzoyl peroxide Microsponges. Benzoyl peroxide (BPO) is primarily used in the treatment of mild to moderate acne. However, its application is associated with skin irritation. It has been shown that encapsulation and controlled release of BPO could reduce the side effect while also reducing percutaneous absorption when administered to the skin. The SEM (Scanning Electron Microscopy) micrographs of the BPO Microsponges enabled measurement of their size and showed that they were spherical and porous. Results showed that the morphology and particle size of Microsponges were affected by drug: polymer ratio, stirring rate and the amount of emulsifier used.

- **Chadawar et.al**[24] Reported Microsponge delivery system. Microsponges are polymeric delivery systems consisting of porous microspheres having a size range in between 5 to 300 micron depending upon the degree of smoothness or after feel required for the end formulations. The present review introduces Microsponge technology along with its synthesis, characterization, programmable parameters and release mechanism of MDS. MDS can provide increased efficacy for topically active agents with enhanced safety, extended product stability and improved aesthetic properties in an efficient and novel manner.

- **Leyden et.al**[25] Carried out comparison of the efficacy and tolerability of once daily tazarotene 0.1% gel and tretinoin 0.1% Microsponge gel were evaluated in a multicenter, double-blind, randomized, parallel-group study in patients with mild-to-moderate inflammatory facial acne vulgaris. Tazarotene was observed to have greater efficacy and comparable tolerability and to be a cost-effective alternative to tretinoin 0.1% Microsponge gel.

- **Shigemitsu Iwai et.al**[26] Reported Biodegradable polymer with collagen Microsponge serves as a new bioengineered cardiovascular prosthesis. Biodegradable materials with autologous cell seeding have attracted much interest as potential cardiovascular grafts. The poly(lactic-co-glycolic acid)–collagen Microsponge patch with and without precellularization showed good histologic findings and durability.

- **Pearl et.al**[27] Performed a Microsponge Formulation of Hydroquinone (HQ) 4% and Retinol 0.15% in the Treatment of Melanoma and Post inflammatory Hyperpigmentation. Microsponges were used to release HQ gradually to prolong exposure to treatment and to minimize skin irritation. The safety and efficacy of this product were evaluated in a 12-week open label study. In this open-label study, micro entrapped HQ 4% with retinol 0.15% was safe and effective.

LITERATURE SURVEYED OF PSORALEN

- **Hsu Y-T et.al**[28] Reported the presence of three isoflavonoid compound in *Psoralea corylifolia*. The method proved to be sensitive, specific, accurate and precise for the determination of daidzein, genistein and biochanin A in *Psoralea corylifolia*.

- **Guo WS et.al**[29] Carried out selection isolation of Iso Psoralen from crude extract of *Psoralea corylifolia*.L by using inclusion method of host guest molecules.

- **Yadava RN et.al**[30] Reported a new biologically active flavonol glycoside from *Psoralea corylifolia*.

- **Liu ZL et.al**[31] carried out extraction and purification of Psoralen from *Psoralea corylifolia*. To establish a method for extracting Psoralen from the seed of *Psoralea corylifolia*.

- **Rajendra Prasad N et.al**[32] Reported antidermatophytic activity of extract from *Psoralea corylifolia* correlated with the presence of a flavonoid compound. The extract showed several degree of antifungal activity against Trichophyton rubrum and Microsporum gypseum the disc diffusion method on a Sabouraud dextrose agar medium.

- **Said.A et.al**[33] Carried out Psoralens percutaneous permeation across the human whole skin and epidermis in respect to their polarity.

- **Arabzadeh et.al.** [34] Reported mechanism of 8 – methoxy Psoralen DNA interaction in the dark. The interaction of 8 – methoxy Psoralen with calf thymus DNA was studied in darkness at 25 ºC and pH 7.4.

- **Said et.al.** [35] Carried out wettability of Psoralen powder influence of bile salts on their contact angles and surface free energy components.

- **Rama Sastry CV et.al**[36] Reported A study of Psoralen photochemotherapy with topical tar in the management of psoriasis vulgaris.

- **Bhatnagar A** [37]et.al Carried out Psoralen and ultraviolet A and narrow-band ultraviolet B in inducing stability in vitiligo, assessed by vitiligo disease activity score: an open prospective comparative study.

- **Gomes AJ** [38] et.al Carried out Identification of Psoralen loaded PLGA poly (DL-lactide-co-glycolide) microspheres in rat skin by light microscopy. Drug delivery systems involving the use of polymers are widely studied and discovery of biocompatible polymers has become the focus of research in this area. Psoralen loaded poly PLGA microspheres to be used in PUVA therapy (Psoralen and UVA irradiation (ultraviolet A, 320-400 nm) of psoriasis were identified in paraffin sections by histological analysis. The Psoralen loaded PLGA microspheres were prepared using the solvent evaporation technique

- **Srinivas CR** [39] et.al Reported Psoralens are chemical compounds derived from certain plants such as ammimajus found in Egypt and Indian plant babachee

which is also called as *Psoralea corylifolia*. Psoralen has been found in more than 30 plants such as lime, lemon, bergamot, parsley, celery, fig and cloves. The medical use of these plants in the treatment of vitiligo by the ancient Egyptians dates back to as early as 1500 B.C. and by the Indians to 1400 B.C.

- **Sergioasffirtri**[40] Reported Reversed phase high performance liquid chromatography determination of lipophilicity of furocoumarins relationship with DNA interaction.

Objective & Plan of work

OBJECTIVE

Leucoderma is a kind of skin disorder and otherwise known as “swetha kuttam” in Sanskrit. It is non infectious disease which occurs due to the deficiency of melanin. In normal condition melanin is produced by melanocytes. Melanin is a complex brown polymer synthesised from amino acid L – Dopa (Dihydroxyphenyl alanine). The initial part of melanin synthesis is catalysed by a copper containing enzyme tyrosinase. This catalyzed the transformation of L – DOPA to tyrosine. Melanin synthesis is under pituitary control (Melanocyte Stimulating Hormone).

Leucoderma can reflect in the mind, causing psychological fears, emotional upset, shame, and socio behaviourial changes by trying to avoid being in the public, attitude of others who insult by avoiding contact. These changes restrict their social behaviour and communications. There may also be fear of ugliness, fear of hereditary (infecting the generation), contagiousness, other diseases, cancer, etc. **Psoralen is one of the drugs of choice for the treatment of Leucoderma over 237 Herbal Drugs.** The dosage forms available in market are having severe side effects.

In the literature survey it is clearly evident that Psoralen Microsponge drug delivery system (MDS) is not yet formulated and my objective is to formulate Psoralen MDS as a topical drug delivery system. Since it is **non-irritating, non mutagenic, non allergic, non toxic and also improves the patient compliance.**

The main objective of my project work is to Isolate the active constituent from crude drug and convert it into Novel Drug Delivery System (Microsponge) to reduce the side effects occurs in available conventional dosage forms. (Psoralen and ultra violet A (PUVA) therapy).

PLAN OF WORK

The scheme of proposed work is as follows

Extraction and Isolation

- Extraction of Psoralen from seeds of *Psoralea corylifolia* – By Soxhlet Extractor.
- Isolation of Psoralen – By Column chromatography

    - Identification of isolated products – By TLC, IR and NMR studies.

Preformulation studies

- Characterization of Psoralen

    - Identification of Psoralen (Chemical Test)
    - Absorption maxima of Psoralen
    - Solubility of Psoralen
    - Infrared study of Psoralen

- Calibration Curve for Psoralen drug

Fabrication and characterization MDS

Fabrication of Microsponge Drug Delivery System

- Polymerization method
- Quasi emulsion solvent diffusion method

Optimization of various parameters in Microsponge formulation

Characterization of Microsponge

- Particle size (SEM) and Shape
- Encapsulation efficacy

Microsponge is incorporate in Gel base

Formulation of gel

Characterization of Gel

- Drug content
- pH
- Viscosity
- Spreadibility
- Tube extrudability

In-vitro studies

Skin irritation studies

Stability studies

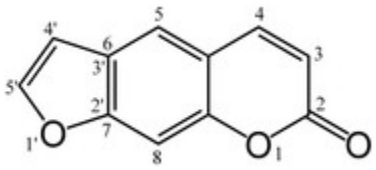
Drug & Excipient Profile

DRUG PROFILE[41]

PSORALEN

In this present investigation, Psoralen was effectively delivered as a Microsponge for the treatment of leucoderma. **Psoralen** (also called **Psoralene**) is the parent compound in a family of natural products known as furocoumarins. It is structurally related to coumarin by the addition of a fused furan ring, and may be considered as a derivative of umbelliferone. Psoralen occurs naturally in the seeds of *Psoralea corylifolia,* it is widely used in **PUVA (Psoralen +UltraViolet A**) treatment for psoriasis, eczema leucoderma and vitiligo.

Structure:



Molecular formula:

$C_{11}H_6O_3$

IUPAC Name:

7H-furo [3, 2-g] benzopyran-7-one

Physical Characteristics:

Brown color or colorless needles, odorless.

Molecular Weight:

186.17 g/mol

Melting Point:

158-161 °C

Category: Topical Pigmenting agent.

Solubility:

Very soluble in chloroform; soluble in ethanol (95%); sparingly soluble in ether.

Practically insoluble in light petroleum (boiling range 60°C to 80°C).

Identification

- **A.** Dissolve 1 mg in 5ml ethanol and add 15ml of a mixture containing 43 volume of water 5 volume of acetic acid and 3volume of propylene glycol a blue fluorescence is visible under Ultra Violet light (365nm).
- **B.** Dissolve 1 mg in 2ml of ethanol and add 0.1M sodium hydroxide a yellow fluorescence is visible under Ultra Violet light (365nm).

Mechanism of action.[42]

Exact mechanism of action in the process of melanogenisis is unknown. The action depends upon the presence of functional melanocytes and their proliferation by the photoactivated Psoralen.

Pharmacokinetics

Oral Psoralen is more than 95% absorbed from GI tract.

Adverse Effect

Overexposure to UV emission may cause severe soreness, redness, blistering or peeling of skin. Swelling of feet, GI upset, nausea, headache and malaise.

Contraindications

Jaundice, Avoid contact with eyelids, genitalia and lips.

Route of administration:

Oral and Topical

Dose:

0.6 to 0.7 mg per Kg per Day.

Use:

Vitiligo, Leucoderma, Psoriasis and Photochemical probe in biological system[42]

ETHYL CELLULOSE [43]

Name

Ethyl Cellulose

Synonyms

Aquacoat ECD, Ethocel, Surelase and Aqualon

Chemical Name

Cellulose Ethyl Ether

Empirical Formula

$C_{12}H_{25}O_6\ [C_{12}H_{22}O_5]_n\ C_{12}H_{23}O5$

Description

Ethyl cellulose is a tasteless, free flowing, white to light tan-colored powder.

Density (bulk)

$0.4 g/cm^2$

Glass transition temperature

129 to 133°C

Solubility

Ethyl cellulose is practically insoluble in Glycerin, Propylene glycol and Water. It is freely soluble in Chloroform, Ethanol 95%, Ethyl acetate, Methanol and Toluene.

Functional Category

Coating agent, Flavoring agent, Tablet binder, Tablet filler and Viscosity increasing agent.

POLY VINYL ALCOHOL [43]

Name

Poly Vinyl Alcohol

Synonyms

Airvol, Vinyl alcohol polymer

Chemical Name

Ethanol, homopolyma

Empirical Formula

$(C_2H_4O)_n$

Molecular Weight

20,000 to 2, 00,000

Description

Occurs an odorless, white to cream colored granular powder.

Melting point

228°C

Solubility

Soluble in water, insoluble in organic solvents.

Application in pharmaceutical formulation

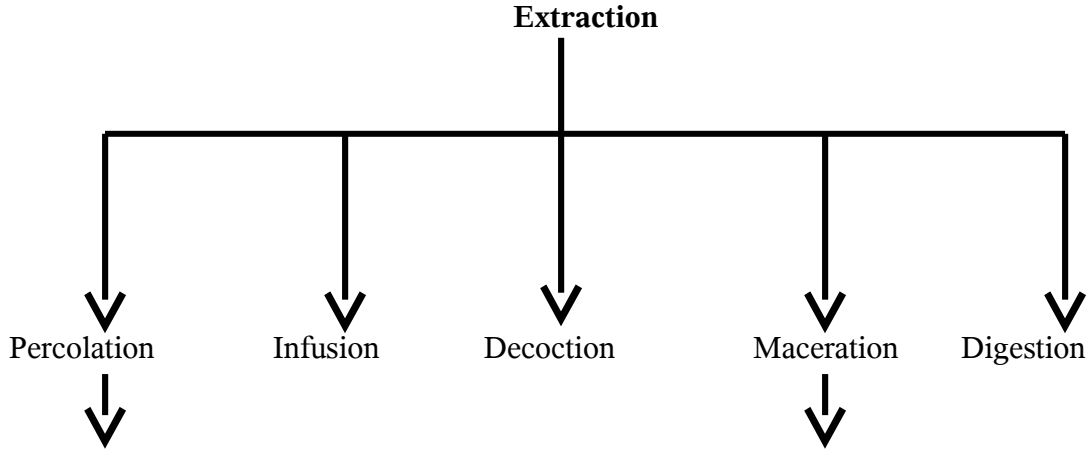
PVA is used primarily in topical pharmaceutical ophthalmic formulation. It is used as a stabilizing agent for emulsion. It is also used as viscosity increasing agent for various formulation such as in sustain release formulation for oral administration[43]

Extraction and Isolation

EXTRACTIION[44]

Extraction is the process of removing or extracting or separating of active constituents from the crude drugs by using suitable solvents such as water, alcohol, solvent ether etc. This is the basic principle involved in extraction process. The active ingredients that have been extracted from the crude drugs are known as extractives and the preparation so obtained are known as extracts.

Fig 2 Types of Extraction



I *Simple percolation*
II *Percolation process for contracted preparation*
III *Continuous hot Percolation*

I *simple maceration*
II *Maceration with adjustment*
III *Multiple maceration*

SOXHLET EXTRACTION

The apparatus consists of boiling flask, soxhlet extractor and a condenser. The drug to be extracted is packed in a filter paper and placed in the extractor. The solvent is taken in the boiled flask. The flask is heated. The solvent is boiled and vapour arises from the solvent and enters in to the condenser through a side tube present in the condenser.

The condensed hot liquid falls on the packed column of the drug and the active constituents are extracted.

As more and more condensed solvent fall on the drug, the level of the solvent in the extractor and the syphon rises. When the level of the solvent reaches the top of the syphon the solvent along with dissolved active constituents drips back into the flask from the extractor through the syphon.

On further heating the vapours of the solvent arises from the flask while the soluble active constituent remains in it and the process is repeated. Every time the solvent drips into the flask from the extractor, the solvent carries more and more active constituent. The same process is repeated again and again until all the active constituents are extracted.

EXTRACTION FOR SEED EXTRACT

100 gm of Psoralen seeds are extracted with ethanol under 60 to 80°C by continuous hot percolation process using Soxhlet apparatus. After completion of extraction. The solvent was removed by distillation under reduced pressure. Brown residue was obtained. The residue is then stored in a desiccator.

ISOLATION OF PSORALEN FROM CRUDE BY COLUMN CHROMATOGRAPHY[45]

Principle of chromatography

Chromatography techniques are used to separate molecules on the basis of difference of size, shape, mass, charge, solubility and adsorption properties. The term chromatography is derived from the Greek meaning coloured writing and was first used by the Russian botanist Tswett to describe the separation of coloured plant pigments on a column of alumina. There are many different types of chromatography

but they all involve interactions between three components: the mixture to be separated, a solid phase and a solvent.

The magnitude of these interactions is dominant in ion exchange chromatography whereas the solute-solvent interaction is more important in partition chromatography.

A brief introduction is given to the theory and practice of these techniques but not all compounds behave exactly as predicted on theoretical grounds and the best conditions for a particular separation often have to be found by practice.

Tab 3 Different techniques of chromatography

Technique	**Solute**	**Solid phase**	**Solvent**
Gel filtration	Size and shape	Hydrated gel	Usually aqueous
Adsorption chromatography	Adsorption	Adsorbent usually inorganic material	Non-polar
Partition chromatography	Solubility	Inert support	Mixture of polar and non-polar solvents
Ion exchange chromatography	Ionization	Matrix with ionized groups	Aqueous buffer

Chromatographic separations can be carried out on a column of material and this is the method used for the isolation and preparation of compounds, alternatively, separations can be made in one or two dimensions on paper or a thin layer and these techniques are used for analytical work

General procedure for running a flash column

Decide on a solvent system and on the quantity of silica

Run TLC's to find a solvent system which will give a good separation of the components of the mixture, and a Rf value of ca. 0.2-0.3 (usually start with ethyl acetate – petroleum ether mixtures). If spots are running close together, an Rf value of 0.2-0.3 at the mid point is normally satisfactory. But if they are well separated, a solvent which puts the lower spot at Rf 0.2-0.3 will usually work. If we know which spot we are most interested in, try to bias our judgment towards this. There are often irrelevant impurities, which are either very polar or very non-polar and these can be largely ignored.

We should try to use as little silica as possible since it is quite expensive. Where the component you require is well separated from other component, as a ratio of Ca 20:1 (silica: mixture) should be sufficient. for more difficult separation up to 100:1 ratio can be used (with a ratio 100: spots that are touching on TLC should be separable in the appropriate solvent system). an important fact to remember for all chromatographic separations is that the more spots there are in the mixture, the greater the ratio of silica for each individual separation. Thus we normally require less silica to separate a 3spot mixture, than for a 2 spot mixture having similar separations. it is in this first step of the procedure that the greatest skill and judgment is required and careful attention should be paid to that TLC's. With experience, the appropriate column and solvent to choose will become almost instinctive.

Choose and prepare the column

Weigh out the required quantity of silica in a conical flask and make it into a mobile slurry with some of the chosen solvent. Choose a column which will fill to about 18cm with the amount of silica being used (it is useful to mark columns once we know how much they hold). To plug the bottom of the column roll a piece of cotton wool between the fingers so that it is wider than the column outlet; connects the column to a low vacuum line with the tap closed: drop the cotton wool ball to the bottom of the column, then open the tap. This is the most reliable way to insert the

plug. Mount the column vertically using a clamp stand, pour about 8cm of the solvent in and then carefully sprinkle a layer of fine sand (ca, 1mm), to cover the plug. Very carefully add the silica slurry to the column, in small portions, via a powder funnel. Between each portion, pressurize the column to pack down the silica and remove excess solvent. Be careful not to allow the solvent to drop below the level of the top of the silica.

When all the silica has been loaded, leave to good head of solvent on top of it and sprinkle in enough sand to cover the surface of the silica evenly (ca. 1mm). Then force the excess solvent through the column until there is just a small layer left above the sand. There should now be a flat layer of silica covered by a thin and even layer of sand.

Load the sample

Dissolve the sample in the minimum amount of solvent, preferably the same solvent that we intend to run the column in (if this is not possible as is often the case, dissolve the sample in a small amount of dichloromethane). Keep a TLC sample of the sample mixture to compare with the column fractions. Load the solution onto the top of the column, very carefully, using a Pasteur pipette to drip it around the walls of the column, just above the sand. Caution must be taken not to disturb the layer of sand. Repeat the procedure using the minimum quantity of solvent to rinse any remaining sample from the flask. Once all the solution has been added allow the level of liquid to drop so that the top of the sand is just starting to dry.

Add the solvents

Add the solvent to the top of the column, very carefully at first, again using a Pasteur pipette to drip solvent around the walls of the column just above the sand, and taking care not to disturb the sand. Once a head of solvent is present, more can be poured in carefully from a beaker. Attach a solvent reservoir to the top of the column and secure it using a Rodaviss collar of elastic bands (Bibby clipps are not strong enough), and fill with solvent.

Run the column

Connect the flash adapter to the top of the reservoir and secure it. Apply pressure to give a fast solvent flow rate and collect fractions continuously. It is very important to maintain a fast flow rate through the column-the solvent should run, rather than drip! A slow flow rate causes reduced resolution NOT improved separation. The size of fractions will depend mainly upon the size of the column and a rough guide, they should be in ml about half the weight of silica (i.e. for a 30g column you should collect ca. 15ml; fractions). It is a myth that we get less mixtures by collecting smaller fractions- the mixture simply appears in more tube and leads to a good deal of extra work. You may feel safer collecting relatively small fractions at first, but as we become more experiences we will tend to collect larger fractions and thus considerably lower the amount of time we spend on chromatography. Always be careful note to let the column run dry. Monitor the column fraction by running TLC'S whilst the column is running (2 to 3 spots per plate is usually a convenient amount). We should have time to apply a spot of the previous fraction to a TLC plate whilst the present fraction's collection.

PROCEDURE FOR PSORALEN SEED EXTRACT

About 45 gm of silica gel (60-120) mesh was loaded in to the glass column using hexane as the solvent by wet packing method. 1.5 gm of the crude Psoralen seed extract was mixed with double the amount of silica gel in a separate Petri dish using Dichloromethane as the solvent. The powdered slurry of drug was passed in to the column and the fractions were eluted using the following solvent ration each fraction was collected with the volume of 20 ml.

Tab 4 Solvent System of Column Chromatography

Fraction No	Solvent Ratio

1	5% Ethyl Acetate: 95% Hexane
2	10 % Ethyl Acetate: 90 % Hexane
3	20 % Ethyl Acetate:80 % Hexane
4	30 % Ethyl Acetate: 70 % Hexane
5	40 % Ethyl Acetate: 60 % Hexane
6	50 % Ethyl Acetate: 50 % Hexane
7	60 % Ethyl Acetate: 40 % Hexane
8	70 % Ethyl Acetate: 30 % Hexane
9	80 % Ethyl Acetate: 20 % Hexane
10	90 % Ethyl Acetate: 10 % Hexane
11	100 % Ethyl Acetate
12	90 % Ethyl Acetate: 10 % Chloroform
13	80 % Ethyl Acetate: 20 % Chloroform
14	70 % Ethyl Acetate: 30 % Chloroform
15	60 % Ethyl Acetate: 40 % Chloroform
16	50 % Ethyl Acetate: 50 % Chloroform
17	40 % Ethyl Acetate: 60 % Chloroform
18	30 % Ethyl Acetate: 70 % Chloroform
19	20 % Ethyl Acetate: 80 % Chloroform
20	10 % Ethyl Acetate: 90 % Chloroform
21	100 % Chloroform
22	90 % Chloroform: 10 % Ethanol
23	80 % Chloroform: 20 % Ethanol
24	70 % Chloroform: 30 % Ethanol

25	60 % Chloroform: 40 % Ethanol
26	50 % Chloroform: 50 % Ethanol
27	40 % Chloroform: 60 % Ethanol
28	30 % Chloroform: 70 % Ethanol
29	20 % Chloroform: 80 % Ethanol
30	10 % Chloroform: 90 % Ethanol
31	100 % Ethanol

THIN LAYER CHROMATOGRAPHY (TLC)

Principle

Thin Layer Chromatography is similar to paper chromatography but the stationary phase is a finely divided sorbent spread as thin layer on a supporting flat plastic, aluminum and glass plate. Solutes migrate through the stationary phase at rate determined by their distribution ratio, those with the large values moving the least if at all, while those with the smaller values moving with the advancing mobile phase/solvent front.

Application of the extract for separation

The extract of elute fraction from the column was taken in a capillary tube and it was spotted on a TLC plate 2cm above its bottom most solution for application were between 0.1 to 1% strength. The starting points equally sized as far as possible and had a diameter ranging from 2-5 millimeter.

The fractions collected from column were simultaneously by analyzed by Thin Layer Chromatography (TLC) using silica gel coated by hand. The pure compounds were identified from TLC using Iodine Chamber method. Similar pure compound were mixed together and the fraction was ready for characterization by Infra Red spectrometer and Nuclear Magnetic Resonance. Before characterization of component the solvent was removed by evaporating in vacuum oven.

PURE COMPONENT OF PSORALEN EXTRACT RESULT

The most biological active prominent pure component was isolated from column chromatography and it was identified as fraction 10-14. After the component is characterized by IR, NMR.

ELUTION[46]

Tab 5 Solvent System of TLC

Plates No	Fraction with crude	Mobile Phase Ratio
1	1-3	30% Ethyl Acetate : 70% Hexane
2	4-6	60 % Ethyl Acetate : 40 %Hexane
3	7-9	75 % Ethyl Acetate :25 % Hexane
4	10-11	85 % Ethyl Acetate : 15 % Hexane
5	12-14	40 % Chloroform : 60 % Ethyl Acetate
6	15-17	60 % Ethyl Acetate : 40 % Chloroform
7	18-19	40 % Ethyl Acetate : 60 % Chloroform
8	20-21	80 % Ethyl Acetate : 20 % Chloroform
9	22-26	75 % Chloroform : 25 % Ethanol
10	25-27	55 % Chloroform : 45 % Ethanol
11	28-30	75 % Ethanol : 25 % Chloroform
12	31-33	100 % Ethyl acetate

Infrared (IR) Spectral Assignment:

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. IR spectrum of Psoralen was taken out

on FTIR (Perkin Elmer Spectrum Rx-1) spectrophotometer. Various peaks in IR spectrum were interpreted for the presence of different groups[51]

Nuclear Magnetic Resonance (NMR)

One useful feature of NMR spectra is that, the more protons there are of a given type, the stronger is the peak in the spectrum. In fact, the area under the peak gives us a measure of how many protons are involved. By using scale of chemical shift we could actually count the number of protons. Fortunately the measurement of the area is done automatically by the spectrometer.

The confirmation of Psoralen has been carried out by NMR spectrometer (Bruker NMR) using deuterated chloroform as a solvent. The final structure of the isolated compounds may posse's basic nucleus Enol (Eu IV), Furan (Eu II) as confirmed by delta values in parts per million (ppm) of ^{1}H NMR spectrum Eu II. The result obtained were presented table 5 for Eu II. [47 – 50]

Fig 3 TLC Analysis of Different Fractions (III & IV)

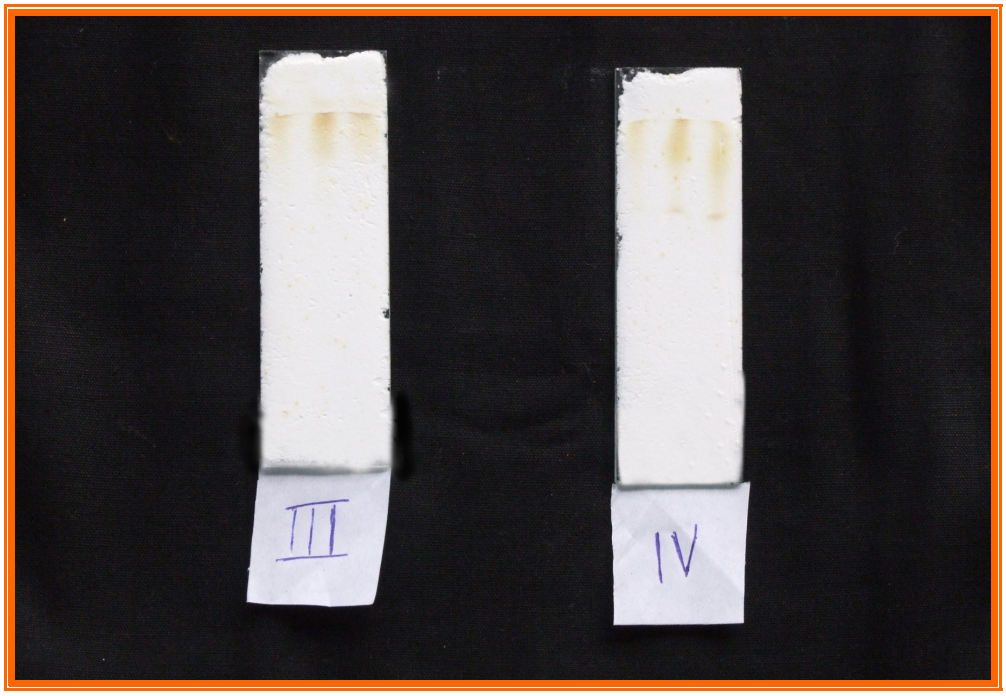
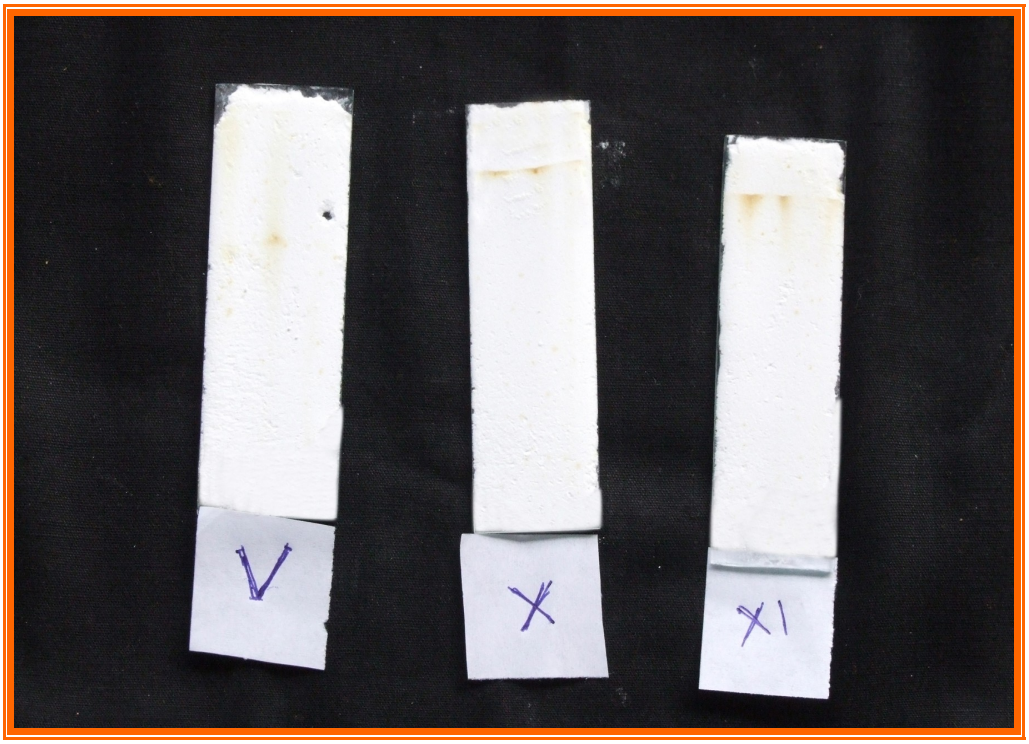


Fig 4 TLC Analysis of Different Fractions (V, X& XI)



Tab 6 Interpretation of IR Spectrum

Functional group	Wave number Cm^{-1} (Centimeter)			
	Crude extract	**Sample II**	**Sample IV**	**Ethyl Cellulose**
- OH	3435.97	3421.85	3397.20	------
-CH-	2927.15	2928.15	2975.91	2 925.54
- CH_2	2365.34	2362.38	2360.57	2362.36
- C_6H_5	------	1692.16	------	1635.45
C=O	1626.88	1613.03	1649.92	1740.93
C=C	1396.23	1423.68	1390.16	1384.56
-C-O-C	1111.23	1179.05	------	1113.21

Tab 7 Interpretation of NMR Eu II

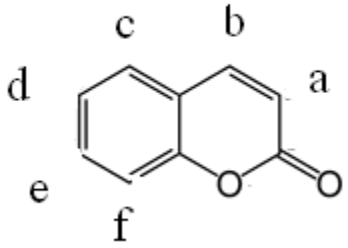
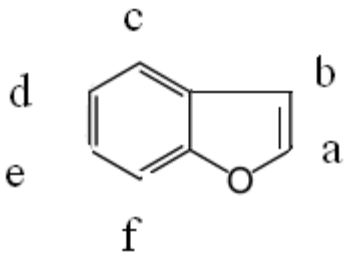
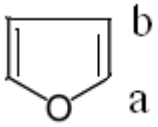
Structure	Delta values in Parts Per Million(ppm)
	a = 7.52 b = 6.66 c = 7.49 d = 7.13 e = 7.19 f = 7.42
	a = 6.45 b = 7.80 c = 7.63 d = 7.22 e = 7.45 f =7.20
	a = 7.38 b = 6.30

Fig 5 Infra Red spectrum of Crude Extract of Psoralen

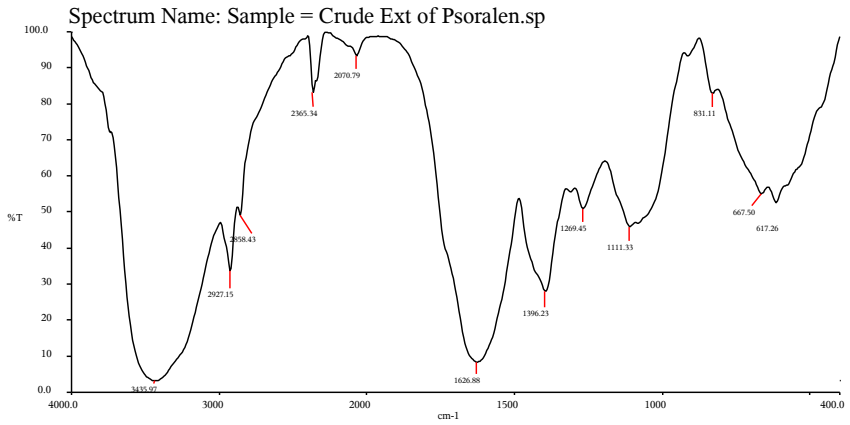


Fig 6 Infra Red spectrum of sample II

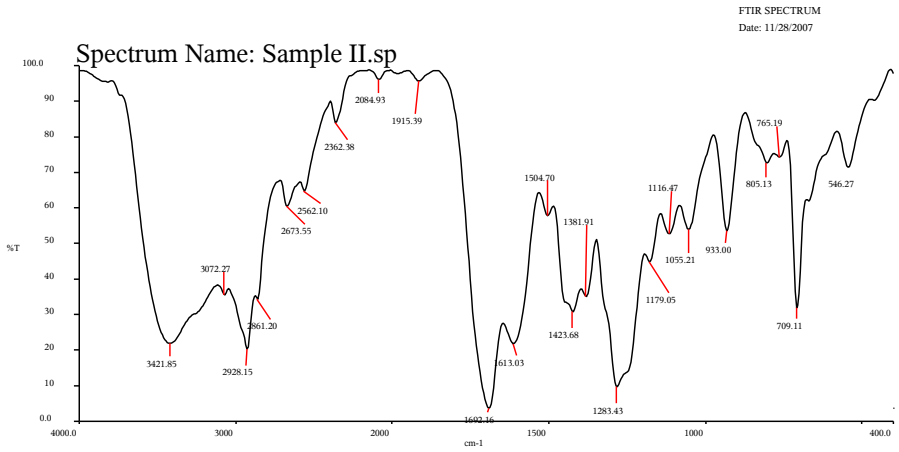


Fig 7 Infra Red spectrum of sample IV

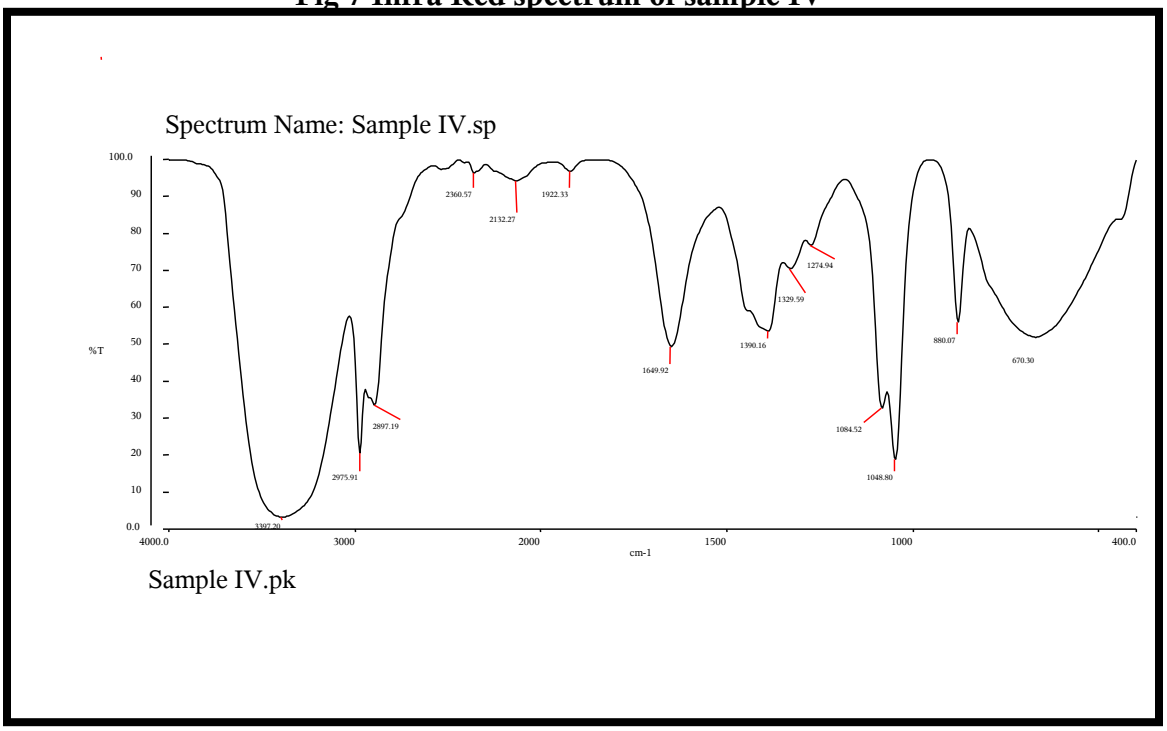


Fig 8 Infra Red spectrum of Ethyl Cellulose

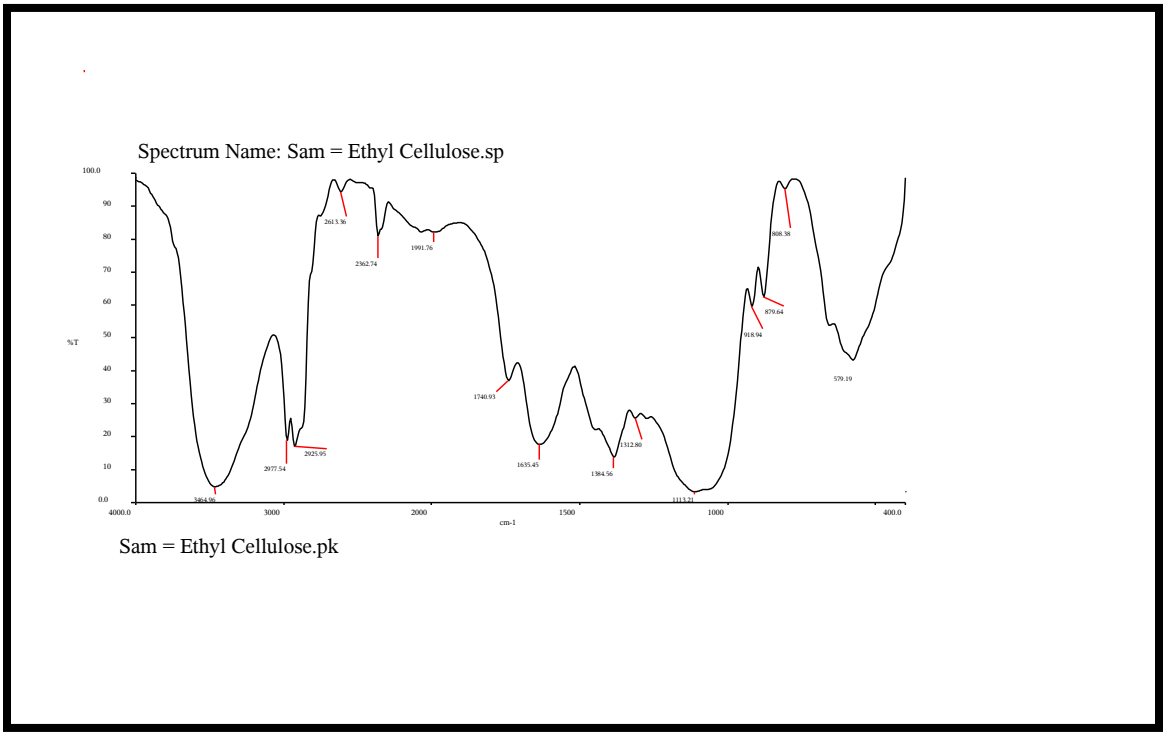


Fig 9 NMR Spectrum of isolated compound Eu IV

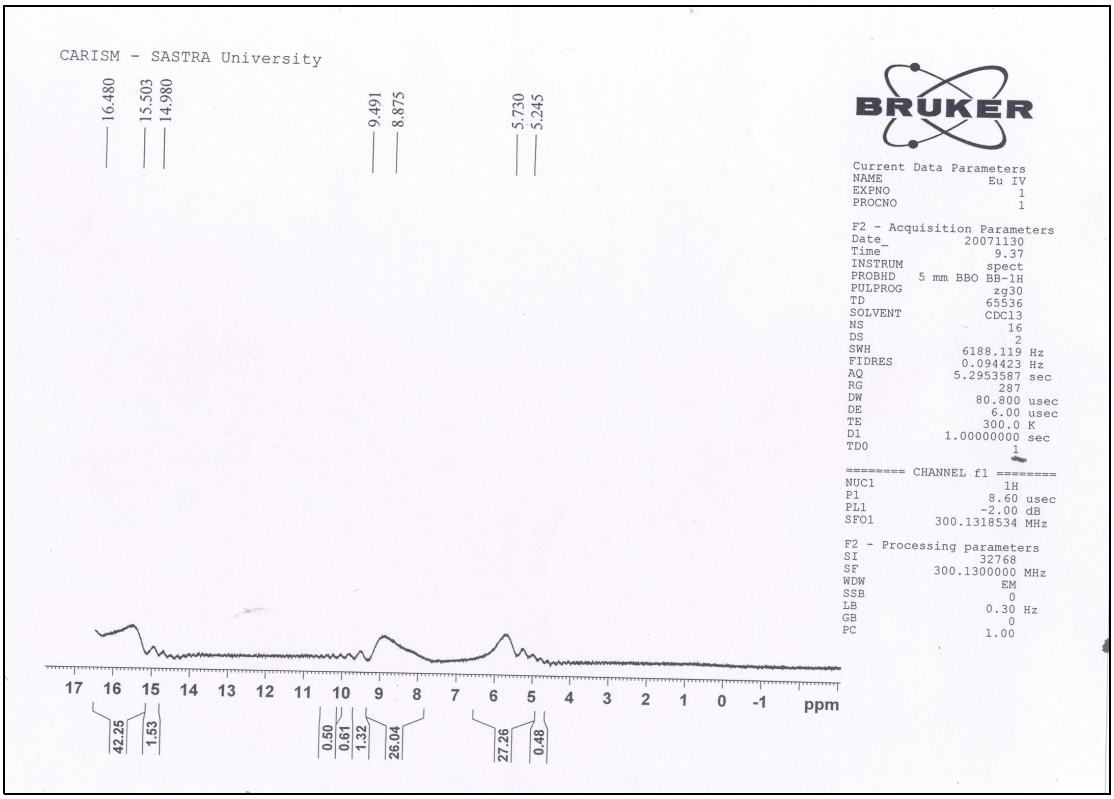
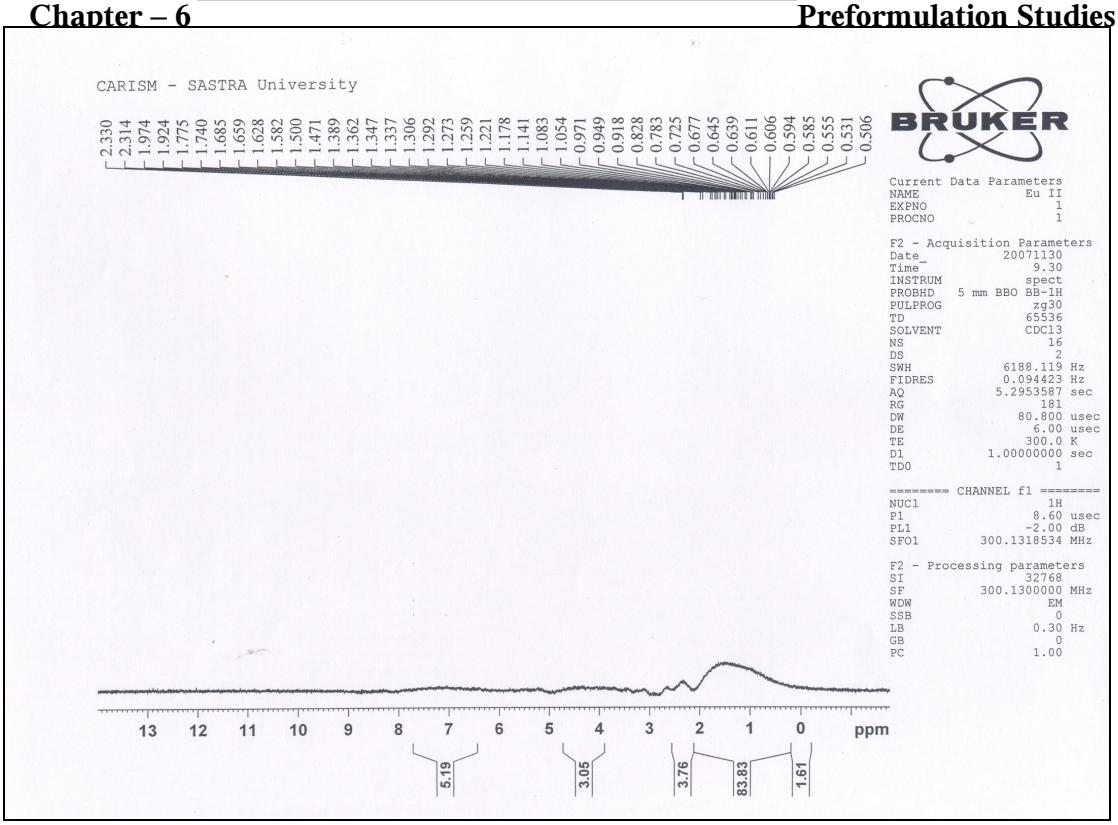


Fig 10 NMR Spectrum of isolated compound Eu II



PREFORMULATION STUDIES

The physiochemical properties of drug plays an important role in the designing of the drug delivery systems. Hence, Preformulation studies are essential to characterize drug for proper designing of the drug delivery systems. The Preformulation studies, which were performed in this project work, include identification of drug (chemical test, UV maxima) and solubility analysis.

DRUG IDENTIFICATION[36]

IR spectra and Absorption maxima identified the isolated of drug from crude extract. (I.P (Indian Pharmacopeia) 1996)

Chemical Test

A. 1 mg isolated drug was dissolved in 5ml ml ethanol and then added with 15ml of a mixture containing 43 volume of water 5 volume of acetic acid and 3volume of propylene glycol. A blue fluorescence was visible under Ultra Violet light (365nm).
B. 1 mg isolated drug was Dissolved in 2ml of ethanol and then added with 0.1M sodium hydroxide. A yellow fluorescence was visible under Ultra Violet light (365nm).

Inference:

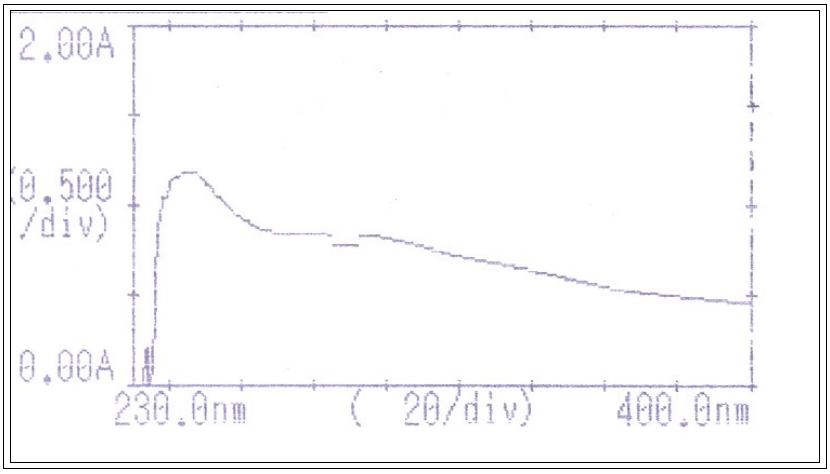
It showed blue fluorescence and yellow fluorescence under Ultra Violet light (365nm). This confirms the drug was Psoralen.

Absorption Maxima

Drug molecule in solution when exposed to light in the visible/ultraviolet region of the spectrum absorbs light of particular wave length depending on the type of electronic transition associated with the absorption.

The drug solution (1mg/ml) in phosphate buffer pH 6.8 was taken in the cuvette scanned in the range of 230 to 400 nm in a UV spectrophotometer. **It exhibits maxima at 247 nm**. Therefore, all the further measurements were taken at 247 nm[36]

Fig 11 Absorption maxima of Psoralen



Solubility Studies

The spontaneous interaction of two or more substance to form homogeneous molecular dispersion is called as solubility. The solubility of Psoralen was studied in various solvents. Psoralen (10mg) was suspended separately in a 10 ml of different solvents at room temperature in tight closed test tube and shaken on wrist action. The solubility of Psoralen in various solvents are follows

A. Very soluble in chloroform; soluble in ethanol (95%); sparingly soluble in ether

B. Practically insoluble in light petroleum.

Calibration Curve of Psoralen

Preparation of phosphate buffer pH 6.8

Phosphate buffer pH 6.8 was prepared according to the following methods (I.P.1996). Disodium hydrogen phosphate (6.8g) was dissolved in 500ml of distilled water and sodium hydroxide (1g) was dissolved in distilled water from that 224ml of

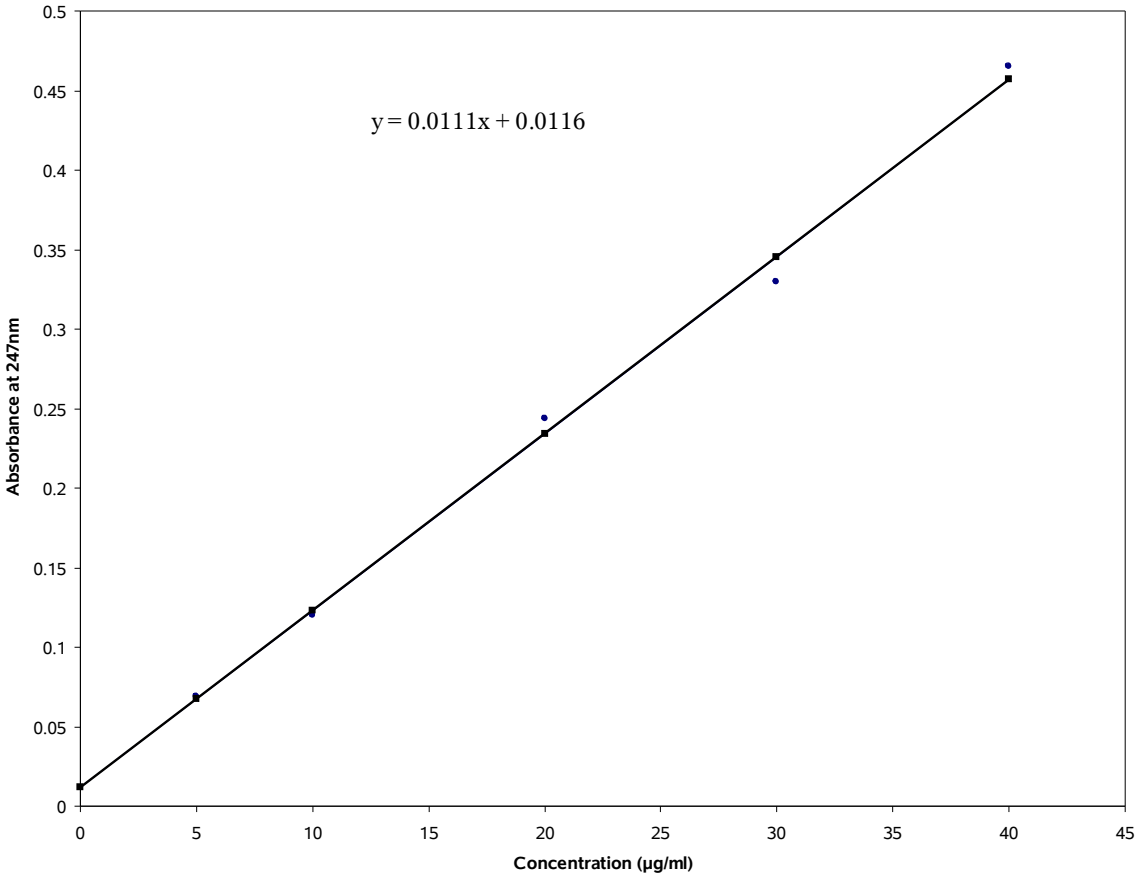
solution was taken and added with above solution and volume was made up to 1 liter with distilled water. The pH was adjusted to 6.8 prior to quantitative estimation[28].

Preparation of Calibration Curve of Psoralen in Phosphate buffer pH 6.8

Accurately weighed quantity (100mg) of Psoralen was transferred into a 100ml volumetric flask and dissolved in small amount of buffer pH6.8 and made up the volume with same buffer to make the standard stock solution of 1mg/ml. From the stock 1ml was taken in 10 ml volumetric flask and made up the volume with the buffer, from this solution 0.5ml to 3ml solution was transferred to 10 ml volumetric flask and made up required volume with buffer and resulting concentration ranging from 5 to 30 micrograms per ml. The absorbance of these solutions was determined at 247 nm using UV spectrophotometer.

The standard curve was constructed between the absorbance and concentration. This standard curve was linearly regressed and statistical parameter related it was derived.

Fig 12 Calibration Curve of Psoralen



Tab 8 Calibration curve of Psoralen

S. NO	Concentration (µg/ml)	Absorbance at 247nm

1	5	0.069
2	10	0.120
3	20	0.244
4	30	0.330
5	40	0.457
r = 0.9979 a= 0.0116 b = 0.011		

Fabrication & Characterization

FABRICATION AND CHARACTERIZATION OF MDS

Psoralen Micro sponges were prepared by Quasi emulsion solvent diffusion method. In this method, the organic internal phase containing **Psoralen** (50mg) and Ethyl Cellulose in 20 ml Dichloromethane was gradually added into 60 ml distilled

water which contained different concentration(0- 2 %) of Poly Vinyl Alcohol (PVA) as emulsifying agent. The mixture was stirred for 6 hours, at 25 °C. The formed Microsponge were filtered and washed with distilled water before being tray-dried at room temperature. For the evaluation of the effect of drug: polymer ratio on the physical characteristics of Microsponge, different weight ratios of drug to Ethyl Cellulose (EC) (1:0.5, 1:1, 1:1.5) were employed. In all these formulations, the total amount of drug was kept constant. To optimize the particle size, size distribution and the drug release from the Microsponge, an individual formulation was selected and a series of the Microsponges were prepared using different stirring rates. However, for optimizing the preparation method and the characteristics of the prepared Microsponge were evaluated[10].

Optimization of various parameters

During the Microsponge preparations, the variables such as amount of polymer, emulsification agent, stirring time, and stirring speed were optimized for getting spherical shape Microsponge with maximum drug encapsulation efficacy.

Tab 9 Optimization of various parameter of the formulation

Formulation & process variables	Drug (mg)	Polymer (mg)	PVA (%)	Stirring Time (hours)	Stirring Speed (rpm)	Encapsulation efficacy ± S.D. (%)

Polymer (*EC*)	50	25	2	6	Medium	53.13±1.5
	50	**50***	2	6	Medium	70.52±0.8
	50	75	2	6	Medium	62.68±1.9
Emulsification agent (PVA)	50	50	without	6	Medium	--
	50	50	1	6	Medium	48.30±0.9
	50	50	**2***	6	Medium	70.52±0.8
Stirring time	50	50	2	2	Medium	55.76±1.2
	50	50	2	4	Medium	59.20±0.8
	50	50	2	**6***	Medium	70.52±0.8
Stirring speed	50	50	2	6	Slow	49.45±0.4
	50	50	2	6	**Medium***	70.52±0.8
	50	50	2	6	High	34.24±0.9

* Optimum parameter, the values are mean ±SD (n = 3)

Tab 10 Optimized formula for MDS

Formulation code	**Polymer concentration (mg)**	**Drug concentration (mg)**	**Stirring speed**	**Stirring time (hours)**	**Encapsulation efficacy ± S.D. (%)**
P - MDS 2 (1:1) ratio	50	50	Medium	6	70.52

Compatibility Study by Differential Scanning Colorimeter (DSC)

Fig 13 DSC of Psoralen

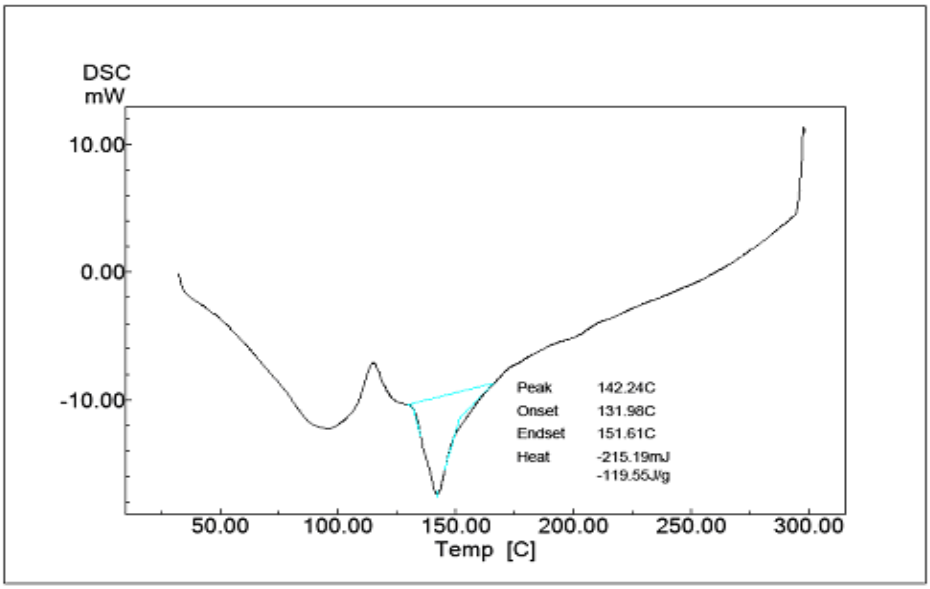


Fig 14 DSC of Ethyl Cellulose

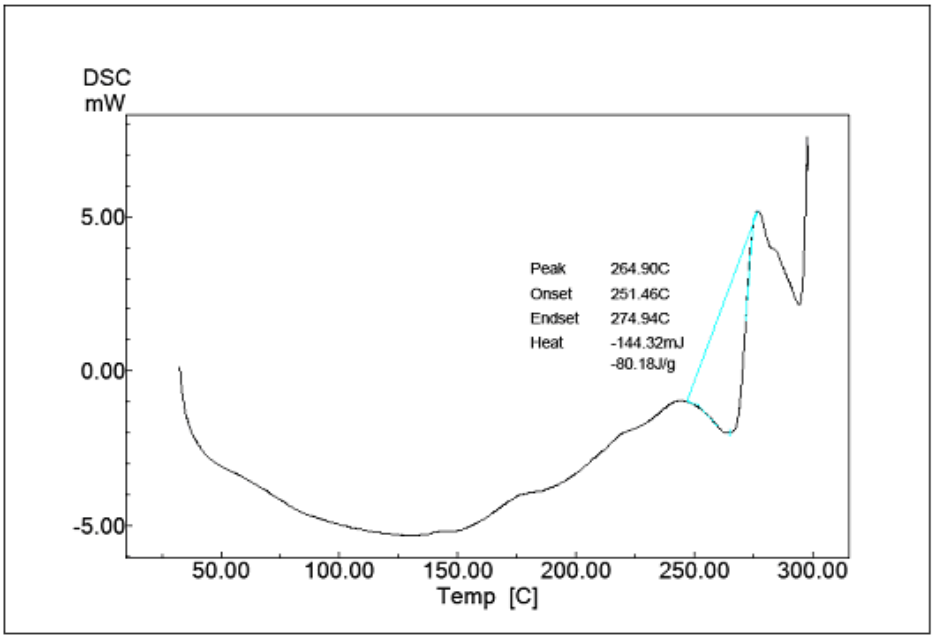
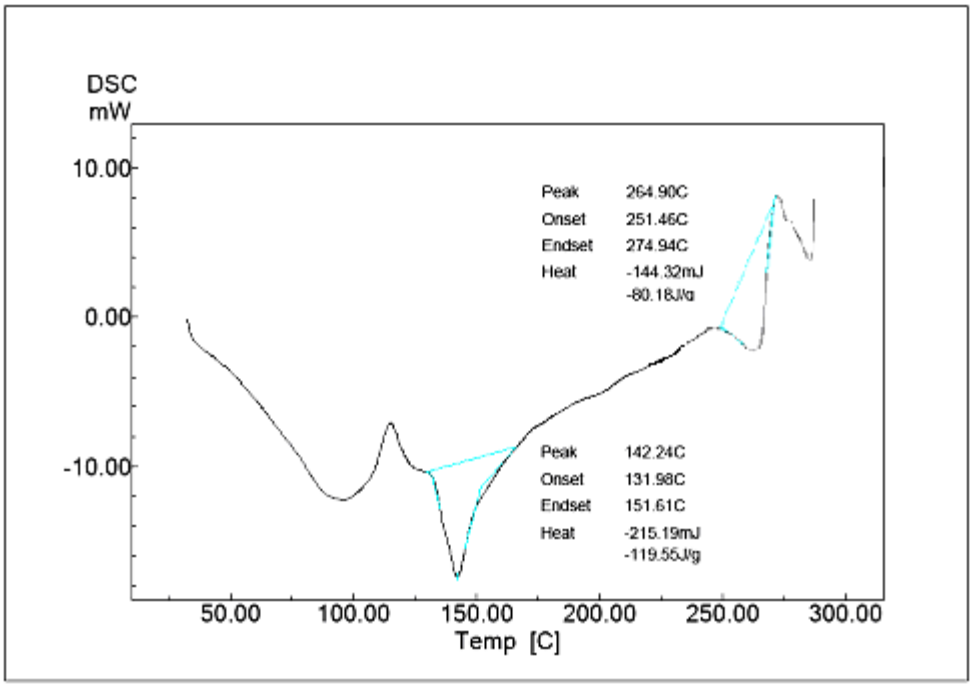


Fig 15 DSC of P – MDS 2



Characterization of MDS

MORPHOLOGICAL STUDY

Prepared MDS was characterized by Scanning Electron Microscopy (Hitachi model S- 30000H, Japan). The MDS was coated with Gold-Palladium alloy. After coating the scanning was taken to determine the particle size and Shape. (Fig 16-19)

DRUG ENCAPSULATION EFFICACY

The amount of Psoralen encapsulated in MDS was estimated by using the following formula.

Amount of drug released from the lysed MDS

Encapsulation efficacy (%) =

Amount of drug initially taken to prepare MDS

Fig 16 SEM PHOTOGRAPHY OF P – MDS 1 (1:0.5)

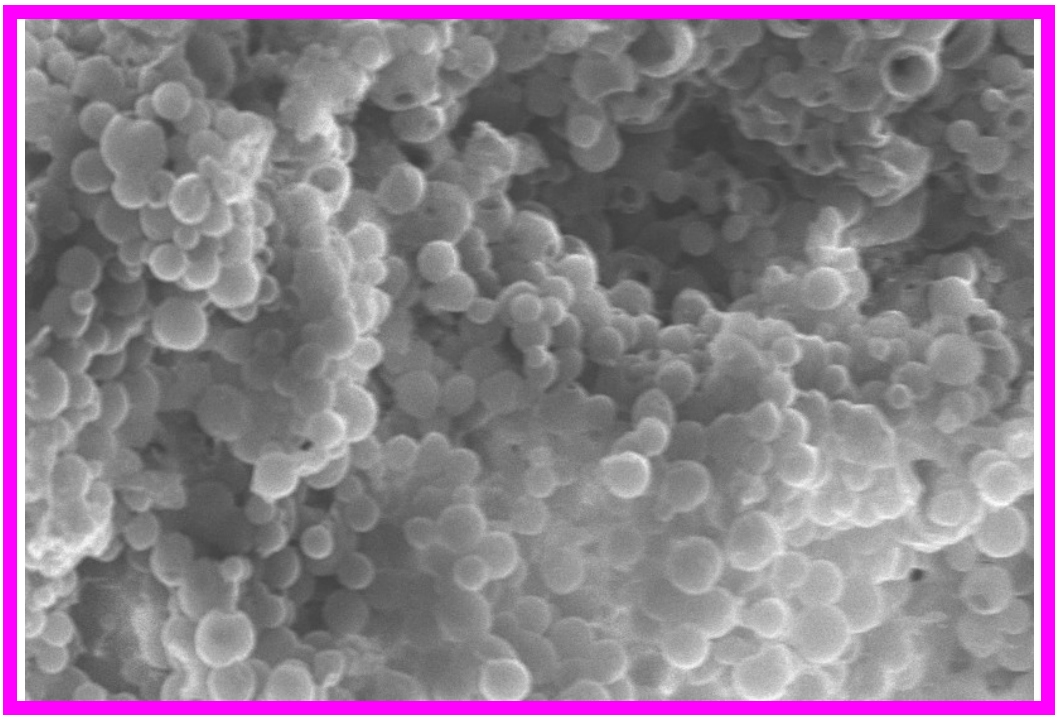


Fig 17 SEM PHOTOGRAPHY OF P – MDS 2(1:1)

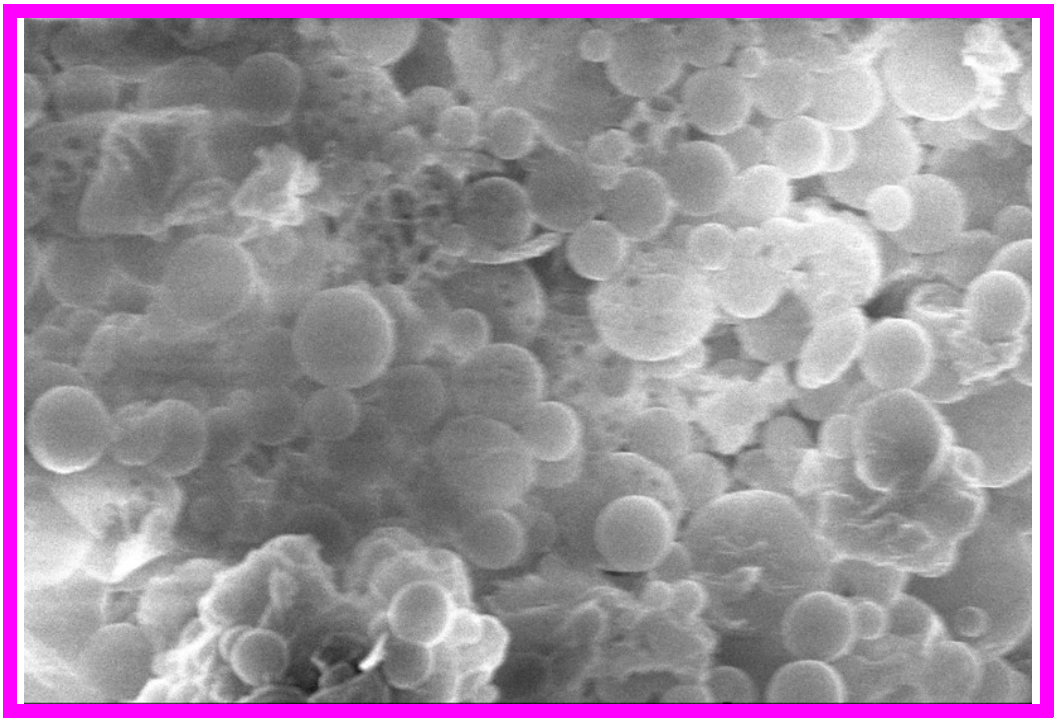


Fig 18 SEM PHOTOGRAPHY OF P – MDS 3(1:1.5)

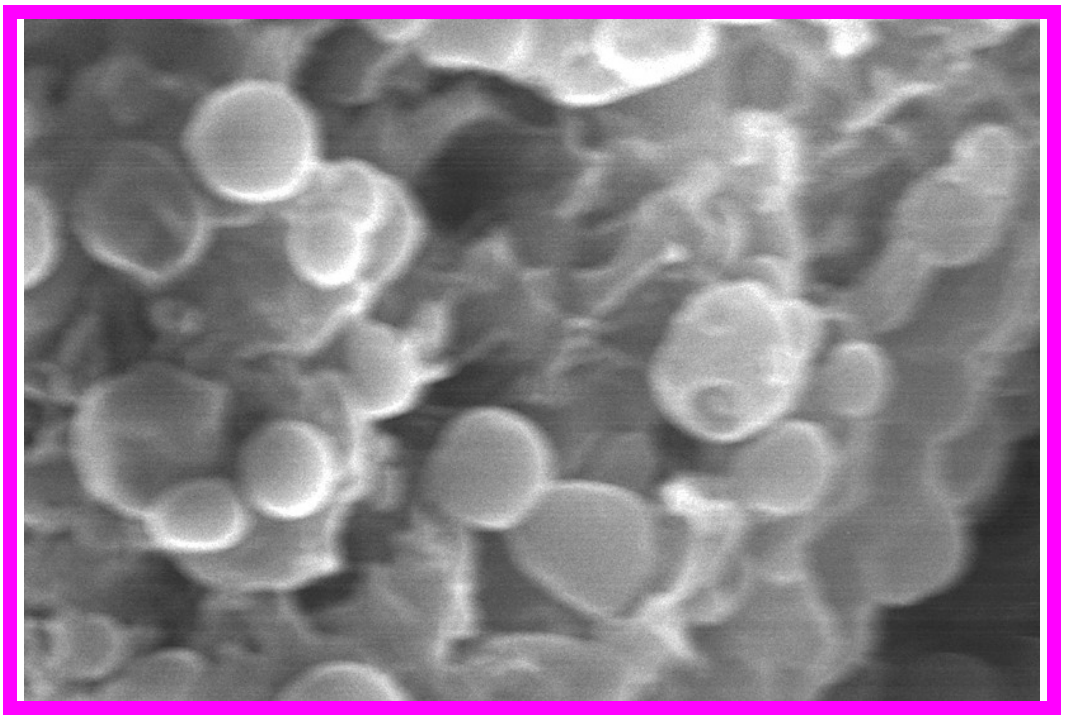
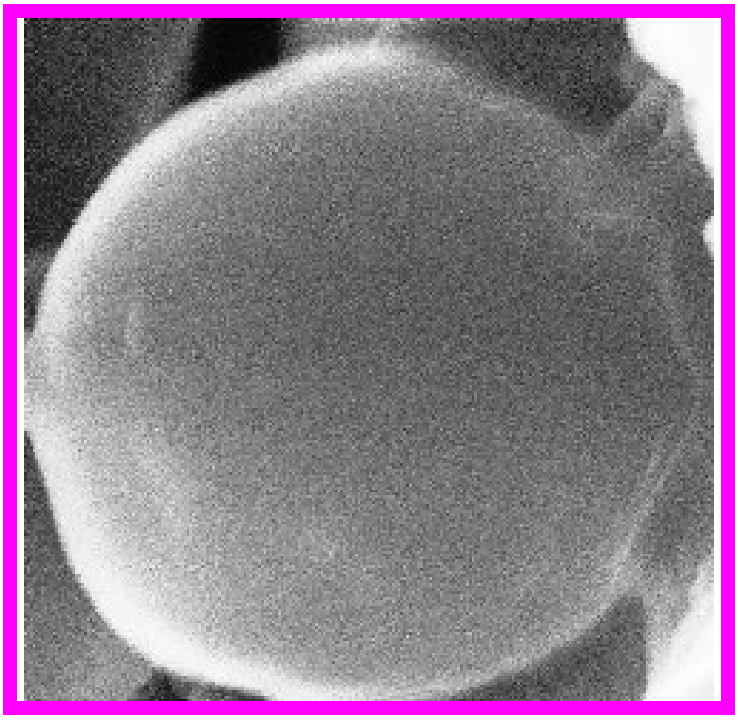


Fig 19 Shape of the Microsponge



MDS INCORPORATED IN GEL BASE

Prepared Microsponges were incorporated in to 1% carbopal 934 Gel base to form a gel and evaluated the following parameters

- pH
- Viscosity
- Drug content analysis
- Spreadability
- Extrudability

pH

pH of the formulation was measured by using a digital type pH meter (Elico Make) by dipping the electrode completely in the Microsponge gel so as to cover the electrode and read out the pH.

Viscosity

The viscosity of the prepared gel was measured using Brookfield viscometer (RV 1 Brookfield Viscometer) at a controlled temperature.

Drug content

A specific quantity (1.0gm) of Psoralen Microsponge gel was dissolved in distilled water and the solution is filtered through the Whatman filter paper. The absorbance of the solution was measured by UV spectrophotometer (Shimadzu 1700) at 247nm against blank.

Spreadability

One of the criteria for a gel to meet ideal quality is that it should possess good spreadability. About 1gm of Psoralen Microsponge gel was weighed and kept at the center of the glass plate (10x10 cm) and another glass plate is placed over it carefully. 2 kg weight was placed at the center of the plate (avoid sliding of the gel). The diameter of the gel in cms, after 30 minutes was measured.

Tube extrudability

It is a useful empirical test to measure the force required to extrude the material from a tube. The formulation under study was filled in a tube with nasal tip of 5 mm opening tube extrudability was then determined by measuring the amount of gel extruded through the tip when a pressure was applied on the tube gel.

Tab11 Evaluation parameter of P-MDS in Gel base

Formulation code	**pH**	**Viscosity Centipoises (Cps)**	**Drug content (%)**	**Spreadability Centimeters (Cm)**	**Extrudability (Grade)**
P-MDS – 1G	6.72	42500	93.04	5.4	E
P-MDS – 2G	6.80	41000	90.16	5.2	E
P-MDS – 3G	6.56	42000	85.52	5.3	G

G – Good E – Excellent P-MDS G - Psoralen Microsponge Drug Delivery System

***In vitro* diffusion studies**

Cellophane Membrane Treatment

Cellophane membrane was boiled in the distilled water for 1 hr and washed with fresh distilled water for three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3% sodium sulphite and soaked in distilled water for 2min at 60°C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study. [52]

Drug Permeation Studies

The *in vitro* release rate of Psoralen were evaluated by open ended tube through the cellophane membrane method using PB pH6.8 as diffusion medium up to 12 hours studies. The cellophane membrane is tided in one end of the tube and then immersed in the receptor compartment containing 200ml of PB pH6.8. Which was stirred at medium speed and maintained at 37°C±2°C. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV1700) set at 247 nm.

Tab 12 Diffusion Profile of P – MDS 1G

S. No	Time in hours (hrs)	Cumulative Percentage drug release P-MDS1G
1	0	0
2	0.25	2.68
3	0.5	3.97
4	0.75	4.08
5	1	8.20
6	2	15.14
7	3	29.88
8	4	34.89
9	5	48.15
10	6	50.86
11	8	54.35
12	10	54.86
13	12	57.52

Tab 13 Diffusion Profile of P – MDS 2G

S. No	Time in hours (hrs)	Cumulative Percentage drug release P-MDS2G
1	0	0
2	0.25	1.27
3	0.5	2.21
4	0.75	3.65
5	1	4.05
6	2	11.25
7	3	14.71
8	4	21.05
9	5	29.09
10	6	42.96
11	8	54.64
12	10	58.68
13	12	61.63

Tab 14 Diffusion Profile of P – MDS 3G

S. No	Time in hours (hrs)	Cumulative Percentage drug release P-MDS3G
1	0	0
2	0.25	0.62
3	0.5	0.94
4	0.75	1.34
5	1	2.17
6	2	3.59
7	3	9.74
8	4	16.95
9	5	29.27
10	6	35.94
11	8	42.24
12	10	44.55
13	12	49.45

Tab 15 Comparative diffusion profile of P – MDS

S. No	Time in hours (hrs)	Cumulative percentage drug release		
		P-MDS1	P-MDS2	P-MDS3
1	0	0	0	0
2	0.25	2.68	1.27	0.62
3	0.5	3.97	2.21	0.94
4	0.75	4.08	3.65	1.34
5	1	8.20	4.05	2.17
6	2	15.14	11.25	3.59
7	3	29.88	14.71	9.74
8	4	34.89	21.05	16.95
9	5	48.15	29.09	29.27
10	6	50.86	42.96	35.94
11	8	54.35	54.64	42.24
12	10	54.86	58.68	44.55
13	12	57.52	61.63	49.45

Fig 20 Diffusion Profile of P – MDS 1G

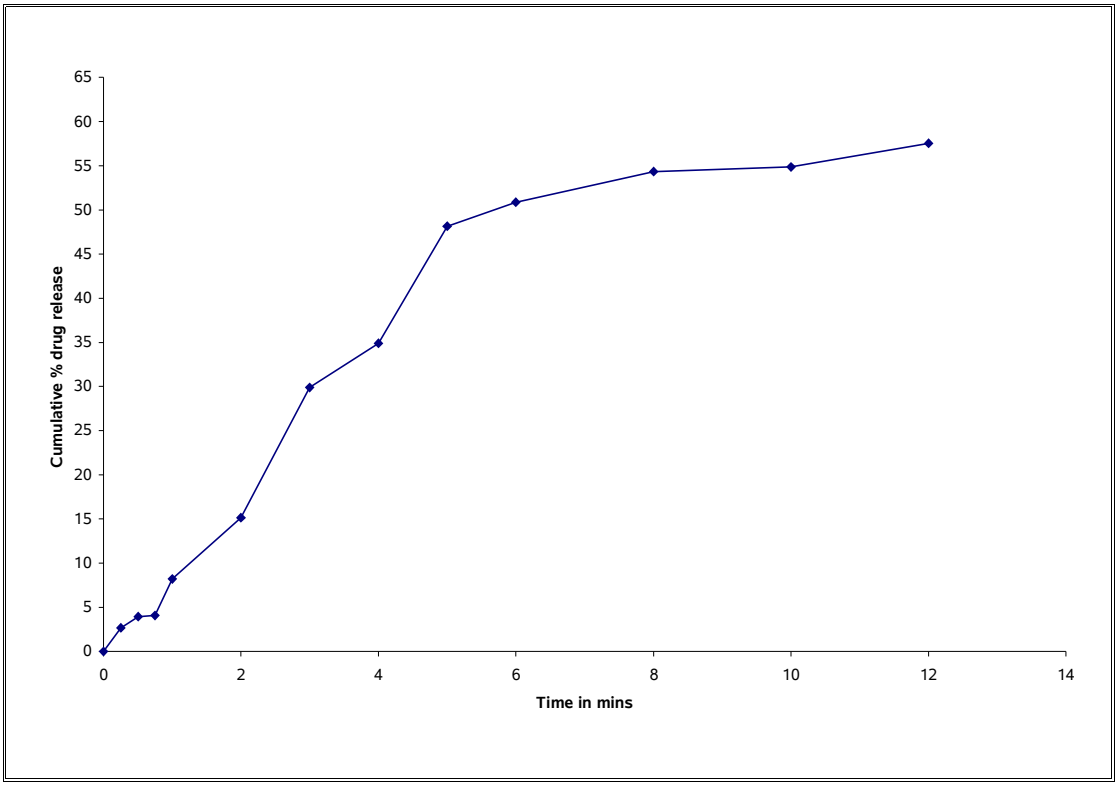


Fig 21 Diffusion Profile of P – MDS 2G

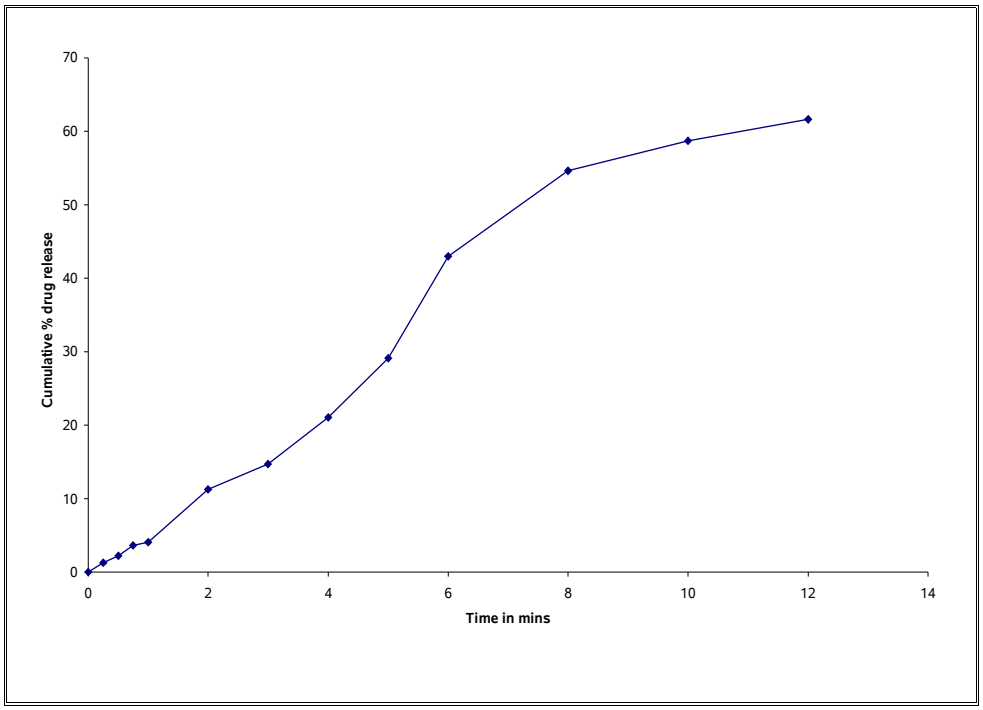


Fig22 Diffusion Profile of P – MDS 3G

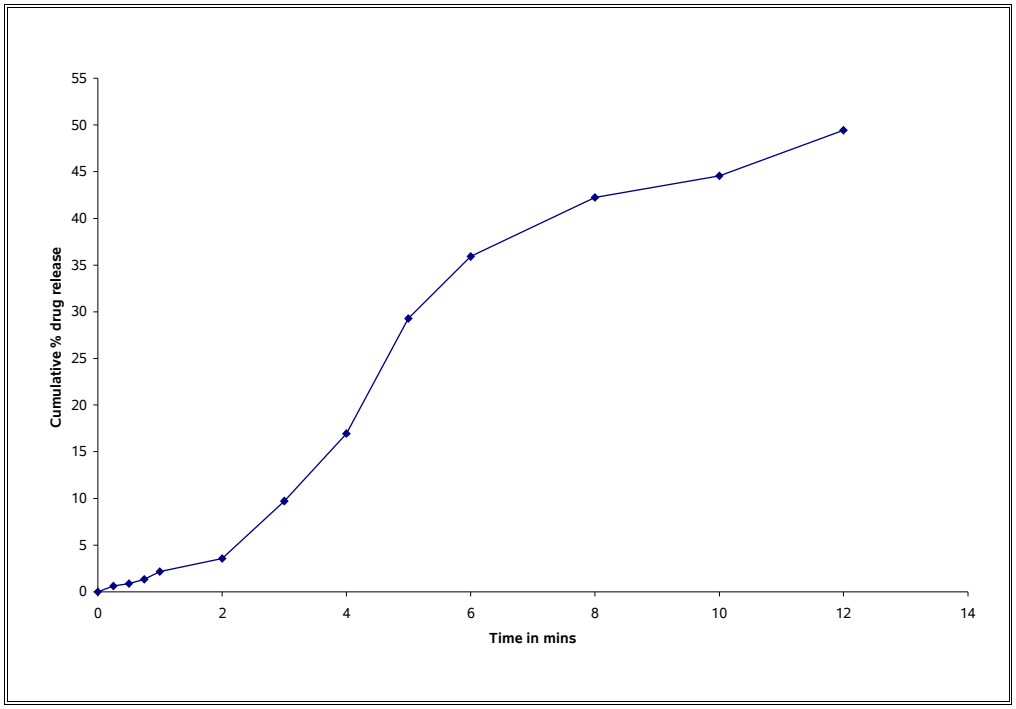
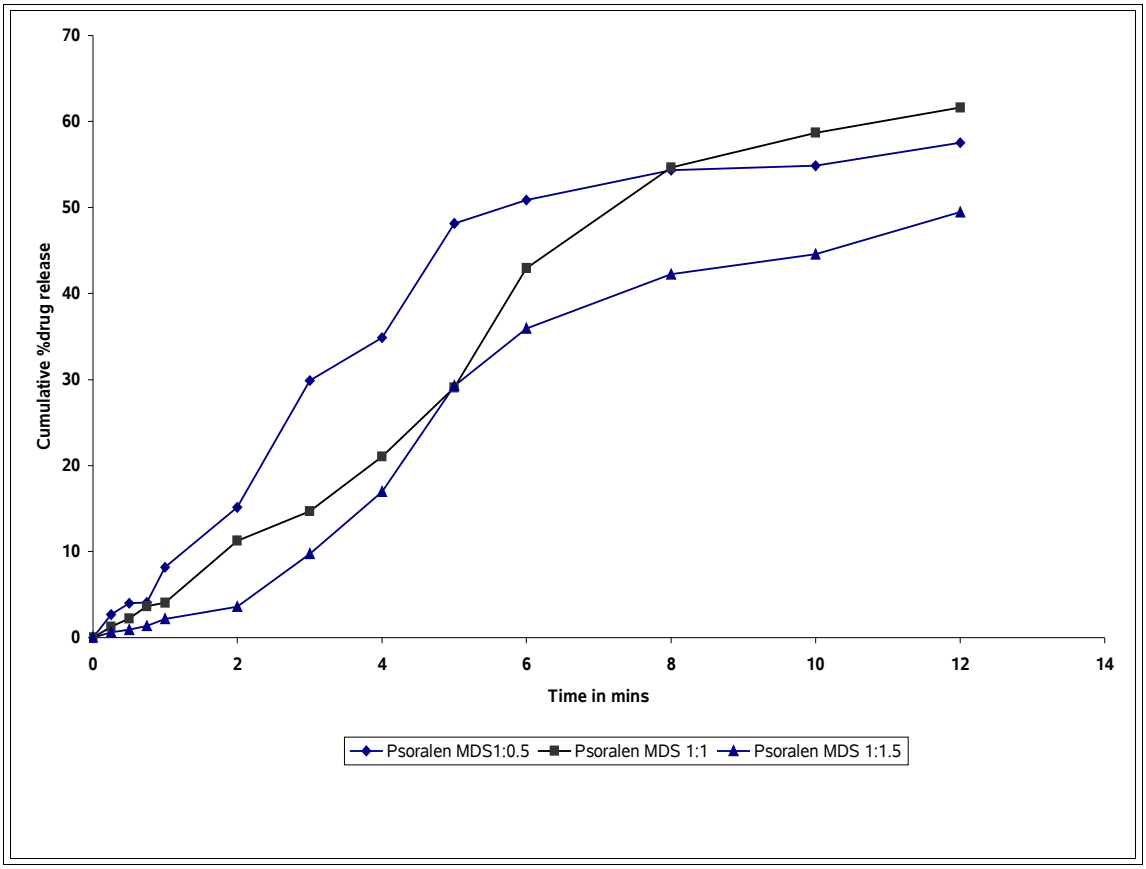


Fig 23 Comparative diffusion profile of P – MDS



Skin Irritation Test

SKIN IRRITATION STUDIES[53]

Skin irritation test was performed on 3 rabbits weighing between 2 – 3.5 kg. Adhesive USP was used as control. The Psoralen Microsponge gel was used as a test sample. Test was conducted unbraided skin of rabbits. The control was placed on left dorsal surface of each rabbit, whereas test formulation were placed on identical side of the right dorsal surface of the rabbits, the gel formulation were removed after 24 hours with the help of an alcohol swab and skin was examined for erythma/oedema.

Tab 16 Skin Irritation Results

Formulation	Skin Irritation
Psoralen in gel base	+
P – MDS 2 G	-

+ indicate edema, - indicate no edema

Inference:

Compare to the formulation P – MDS 2G with Psoralen in gel base, Psoralen in gel base produced erythma and inflammation at the level of 2 millimeter after 24 hours studies.

Fig 24 Preparation of skin irritation test

Fig 25 Control for Skin irritation test (USP adhesive tap)

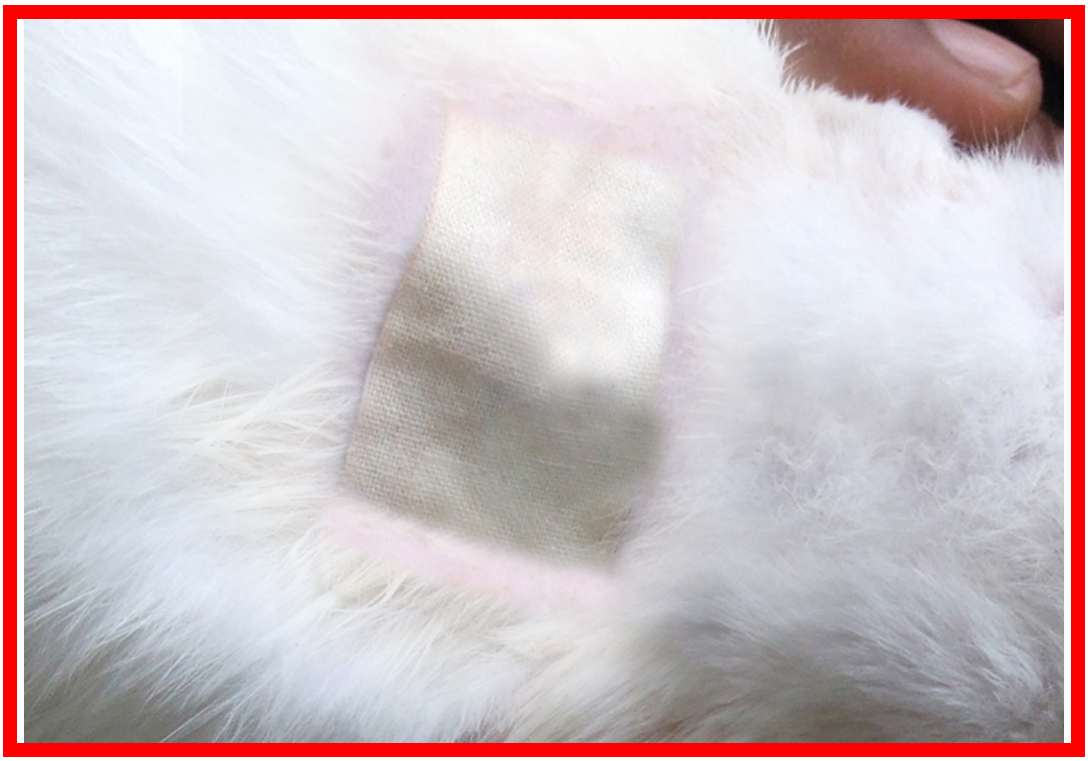


Fig 26 After 24 hours study with P – MDS 2 in Gel base

Fig 27 After 24 hours study with Psoralen in Gel base

Stability Testing

STABILITY STUDIES

In pharmaceutical sense, stability is technically defined as the capacity of particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specification. Durability of a product may be defined as the capability of a particular formulation in a specific container to remain with the physical, chemical, microbiological, therapeutic and toxicological specification. Stability of Microsponge gel formulation on storage is of a great concern as it is the major resistance in the development of marketed preparations. The prepared formulation was tested for stability on storing them at 4±1°C 25±2 °C and37± 5°C & RH (Relative Humidity) 75%

Formulation was stored in gel tube at 4±1°C 25±2 °C and 37±5 °C for a period of three months. After one month and three months they were evaluated for the following parameters:

- Appearance
- pH
- Drug content analysis
- Spreadability

Tab 17 Stability study data of formulation stored at 25±2 °C

Parameter Detected	Duration of Evaluation		
	Initial	After 1month	After 3months
Appearance	Good	Good	Good
pH	6.72	6.78	6.78
Drug Content (%)	90.88	89.36	89.36
Spreadability (cm)	5.4	5.4	5.2

Tab 18 Stability study data of formulation at 4±1°C

Parameter Detected	Duration of Evaluation		
	Initial	After 1month	After 3months
Appearance	Good	Good	Good
pH	6.72	6.66	6.6

Drug Content (%)	90.88	90.16	89.36
Spreadability (cm)	5.4	5.2	5.3

Tab 19 Stability study data of formulation stored at 37±5°C RH 75%

Parameter Detected	Duration of Evaluation		
	Initial	After 1month	After 3months
Appearance	Good	Good	Good
pH	6.72	6.78	6.70
Drug Content (%)	90.88	89.44	89.44
Spreadability (cm)	5.4	5.8	6.1

Stability testing were carried out by exposing formulated P–MDS 2G at various temperature of 4±1°C, 25±2°C and 37±5°C for the period of three months. The results indicated that they are stable both physically and chemically at 4±1°C and 25±2°C**.** In all these 3 temperature, there was no marked change in drug content, pH and physical appearance. The spreadability showed a significant change at elevated temperature 37±5°C**.** Since spreadability was increases with the decrease in viscosity of gel base. Thus it is advisable to store at below 37±5°C.

RESULTS AND DISCUSSION

Leucoderma is a miserable acquired skin disorder making skin white due to loss of melanin pigment. Different plants (*237*) were being used to treat this disorder. The drug of choice for this is Psoralen – isolated from *Psoralea corylifolia.* In the present study Psoralen is considered to design as a Microsponge Drug delivery System (MDS).

ISOLATION AND IDENTIFICATION

Psoralen is extracted from *Psoralea corylifolia* plant seeds by using soxhlet extractor and isolated through column chromatography. After isolation the isolated pure compound was primarily identified by Absorption maxima, IR spectroscopy (Infra Red) and confirmed by Nuclear Magnetic Resonance (NMR) study and the isolated product was brown colour powder, which is used for further work.

The present work emphasis was conversion of crude drug in to Novel drug delivery system – Microsponge Drug delivery System (MDS). The Novel system reduces the side effects which occur in the marketed product especially in Psoralen and Ultra Violet A (PUVA).

The absorption maxima of Psoralen in phosphate buffer pH 6.8 was measured by UV- visible spectrophotometer against the blank and was found to be 247 nano meter (nm). The result was shown in **Fig 11**.

Solubility studies of Psoralen in different solvent at room temperature indicated that the drug was freely soluble in chloroform and soluble in ethanol.

OPTIMIZATION OF VARIOUS PARAMETERS

The Microsponges were prepared by using Quasi – Emulsion Solvent Diffusion Method. The various formulation parameters used in trial formula were

- Polymer concentration
- Emulsifying agent
- Stirring speed and Time

The quantity of polymer used in the preparation was 25 – 75 milligram (mg). The particle size increased due to increase in the concentration of the polymer. The theoretical drug loading was kept constant but the amount of polymer was increased from 25 – 75 mg. The encapsulation efficacy was higher with 50 mg of polymer and got desired size and shape.

The concentration of the emulsifying agent Poly Vinyl Alcohol (PVA) from 0 – 2 % were used to access the effect on the formulation characteristic. The

formulation without PVA were not formed Microsponges. When the concentration of PVA was 1 – 2% give required particle size range [5 – 300 micrometer (mm)] and the encapsulation efficacy was high. The optimized concentration of PVA was found to be 2% in the formulation and hence it forms Microsponges with desired size.

At medium speed, the Microsponges were produced of desired range with maximum encapsulation efficacy. At low speed the Microsponges were not in uniform size and encapsulation efficacy was also less. At higher speed the particle size was small with low encapsulation efficacy.

The stirring time was optimized to get desired shape and higher encapsulation efficacy. The optimized stirring time was 6 hours.

Based on these three parameters formulation P – MDS 2 have desired size, shape and high encapsulation efficacy. The results are shown in **Table No 9**.

COMPATIBILITY STUDY

Compatibility study was accessed by Differential Scanning Colorimeter (DSC). The results are shown in **Fig 13 to 15**. It indicated that there is no chemical interaction between the drug, polymer and MDS.

The DSC thermograms of drug Peak at 142.24, Ethyl Cellulose polymer Peak at 264.90. The thermograms had shown an endothermic peak at 151.6 and 251.46. The DSC thermograms indicated that there is no drug – polymer interaction in P – MDS 2 formulation due to no change in the endothermic peak in the thermogram.

MORPHOLOGIAL STUDY

Scanning Electron Microscopy (SEM) indicated that the Microsponges are in spherical, discrete particle with internal voids with a size range of 5 to 40 µm (micro meter). The results are shown in **Fig 16 to 19.**

IN VITRO DIFFUSION STUDY

In vitro diffusion study was carried out using an artificial membrane for a period of 12 hours. Comparative study of the diffusion profile of the drug from 3 prepared formulations with various drug – polymer ratio are tabulated in **Tab 12 to 15** and presented in **Fig 20 to 23**respectively

A maximum percentage of **61.63%** was obtained from formulation P –MDS 2G during 12 hours study. Therefore the formulated P –MDS 2G was selected for further studies.

SKIN IRRITATION TESTING

The skin irritation studies results are shown in **Fig 24 to 27**. It revealed that the P – MDS 2G formulation do not cause any noticeable irritation or edema in rabbit skin compared to control (USP adhesive tap), but Psoralen in gel base carbopal 934 which causes noticeable irritation and edema compared with control.

This indicates that the P – MDS 2G was safe for topical application for treatment of Leucoderma.

STABILITY TESTING

Stability testing were carried out by exposing formulated P–MDS 2G at various temperature of 4±1°C, 25±2°C and 37±5°C for the period of three months. The results indicated that they are stable both physically and chemically at 4±1°C and 25±2°C**.** In all these 3 temperature, there was no marked change in drug content, pH and physical appearance. The spreadability showed a significant change at elevated temperature 37±5°C**.** Since spreadability was increases with the decrease in viscosity of gel base. Thus it is advisable to store at below 37±5°C.

CONCLUSION

About **3 – 5%** of the world populations are suffering from the skin disorder Leucoderma. Psoralen is a drug choice for treating the skin disorder. Psoralen Microsponges were prepared by **Quasi – Emulsion Solvent Diffusion Method utilizing Ethyl Cellulose as a polymer and** incorporated in gel base.

The present work emphasis was conversion of crude drug in to Novel drug delivery system – Microsponge Drug delivery System (MDS). The Microsponges have a distinct advantage over the existing conventional dosage forms, for example PUVA therapy having severe side effects like **skin -irritation, mutagenic, skin allergic, toxic and blisters etc.**

The prepared P – MDS G has achieved the objective of non – irritation, non – toxic and sustained release and thus improved patient compliance.

Further studies will be carried out to determine the *In Vivo* characteristic P- MDS G.

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